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# SOIL SCIENCE

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TECHNIQUES IN GREENHOUSE STUDIES  
OF  
SOIL DEFICIENCIES AND PLANT NEEDS

This number of *Soil Science* is devoted exclusively to papers that are concerned with the growing of experimental plants, primarily under greenhouse conditions. The papers were prepared by invitation, the authors having been selected by a committee of their colleagues. The thought was to present as wide a variety of procedures as possible and thus give the readers of the Journal the opportunity to understand the many different techniques employed in overcoming the difficulties that are involved in growing plants for research purposes.

No doubt many additional plant scientists could have made equally important contributions to this number. We shall be glad to hear from any of those who employ other techniques and, if sufficient interest develops, possibly a second issue can be devoted, in due time, to this very important topic.

The editor wishes to express his appreciation for the time and thought given by the contributors and for the careful manner in which their papers have been prepared. It is believed that many graduate students, and the men directing their researches, will find themselves greatly indebted to these authors for having joined in this undertaking.

FIRMAN E. BEAR



# GROWING PLANTS IN SAND CULTURES FOR EXPERIMENTAL WORK<sup>1</sup>

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The sand-culture method consists essentially of growing plants with their roots in contact with relatively inert solid particles which are kept bathed with an aqueous nutrient solution, ordinarily of inorganic salts. There are many variations of this method, depending on the species of plant to be grown, the purpose for which the plants are grown, the relative degree of nutrient control that is to be exercised, the physical equipment and environmental conditions available, and the ingenuity of the investigator.

It is not the purpose of this discussion to record all the variations of the sand-culture method, but rather to describe certain equipment and to discuss briefly in comparison with several other techniques some of the factors concerned with a technique of continuous solution renewal which has proved satisfactory in this laboratory for the production, in individual sand cultures, of plants excellent in size, productivity, and sensitivity to environmental changes induced by the investigator. Further discussions of the history, principles, and methods of study of plant nutrition are given elsewhere (10, 21, 22, 24, 25, 36, 49, 55, 56, 61).

## CHOICE OF THE PLANT SUBJECT

Much of the success of an experiment in plant nutrition depends on the choice of a suitable plant subject. Plant species differ greatly in their responses to different environmental conditions such as light intensities, photoperiods, and temperatures, and each species is best grown in an environment that will introduce no undesirable limiting factor in the experiment. For example, the soybean is much more sensitive to changes in photoperiod than is the tomato and consequently it is less suitable for many nutrition experiments during the short winter days of this latitude. The cucurbits are less suited to experimental growth during the winter season than are wheat, oats, lettuce, or radish because of the relatively low seasonal light intensity and temperature levels which usually are present.

Consideration can also be profitably given to differences between varieties or even strains of the same species in the choice of a plant subject. For example, if tomato fruiting and seed formation is the response desired during the winter season, the Michigan State Forcing, a greenhouse forcing variety, is more likely to set fruit as a result of pollination than is a more vigorously vegetative variety such as Rutgers, which, on the other hand, may be the more prolific producer during the summer season.

Choice of a plant subject may well take into account the relative sensitivity

<sup>1</sup> Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, department of plant physiology.

to variations in the nutrient supply. For example, with a given low nutrient level of boron, boron-deficiency symptoms develop to a much less degree with monocotyledons like corn and oats, which have relatively low boron requirements (39), than with dicotyledons like sunflower, tomato, and alfalfa, which have higher boron requirements.

The nature of its genetic constitution is an important consideration in the choice of a plant subject. Some variations between seed-propagated individuals are to be expected, but such variations may be very great particularly if the flowers of the species cross-pollinate readily and the ancestry of the seed is unknown. Selecting seed from successive generations of single self-pollinated tomato plants has yielded seedlings of a high degree of vegetative and physiological uniformity. Still greater uniformity in individuals can be obtained by asexual than by sexual propagation. Rooted cuttings of a single clone of common white clover have been found to have a far higher degree of uniformity with respect to physiological responses to changes in environmental factors than plants grown from seed produced by chance cross-pollination. Rooted cuttings of single alfalfa and tomato plants have likewise proved to be of high uniformity. Any increase in the genetic uniformity of plants permits the use of a smaller number of replicates of cultural treatments without loss of statistical significance of the data than is possible with plants produced from the seed of parents of markedly dissimilar genetic constitution. This becomes an important factor to be considered where greenhouse space and equipment are limited and numerous experimental treatments are to be conducted. Naturally enough, studies involving seed germination and seedling growth preclude the use of rooted cuttings.

#### CULTURE EQUIPMENT

Several systems of sand-culture equipment have been used by various investigators. Each has advantages and disadvantages from the standpoint of maintaining an adequate nutrient supply for the plants and the practicality of its use. Crocks without bottom openings have been used (37, 50) but require considerable attention for good results. Culture drainage systems provide for the distribution, collection, and in some cases the reuse of nutrient solutions for either single or multiple cultures (14, 17, 43). Autoirrigation equipment (30, 42) and flower pots placed in enamel pans (40) provide for a reservoir of nutrient solution available by capillarity. But culture vessels which provide for free bottom drainage have been in most common use. Those of deep conical shape (4) provide zones of widely differing moisture content but are somewhat awkward to use. Those approximately equidimensional and either slightly conical or cylindrical in shape with round, sloping, or flat bottoms (54, 55, 57) have proved generally satisfactory if they have a usable capacity of approximately 5 or more liters.

Simple culture equipment has many advantages over complicated equipment. Complicated apparatus requires considerable time to devise, set up, keep clean, and dismantle and is more difficult to move about the greenhouse than are simple individual culture units. Surfaces in contact with nutrient solutions that are

exposed to light support the growth of algae and require periodical cleaning. The equipment illustrated in figure 1 is the simplest satisfactory combination of individual pieces of apparatus that has been found available for growing plants in individual movable units. Details of some of the components of this equipment have been given previously (55).



FIG. 1. EQUIPMENT COMPRISING A MOBILE UNIT FOR GROWING PLANTS IN SAND CULTURE BY THE CONTINUOUS SOLUTION RENEWAL METHOD

A—culture vessel; B—white quartz sand; C—nutrient solution reservoir bottle and D—dish; E—nutrient solution delivery tube; F—reservoir support; G—culture vessel support; H—plant support; and I—waste pan.

#### CULTURE VESSEL

The ceramic material that has been found most satisfactory for culture vessels is a high-grade white porcelain with an over-all silica glaze.<sup>2</sup> This material is extremely inert chemically, is easily cleaned, is a good reflector of solar radiation, is very resistant to fracture, and does not craze or develop fine cracks. If fine cracks develop in crockery, salts may penetrate beneath the surface and during subsequent use become a source of contamination. The culture vessel illustrated (fig. 1.1) is a flower pot of standard design and composed of this porcelain

<sup>2</sup> Manufactured by Trenton Potteries Company, Trenton, New Jersey.

material. Pots of this type have been used to study the effects of nutrient deficiencies of iron, manganese, and boron as well as of those chemical elements used by plants in relatively large amounts. These pots have an outside top diameter of about 23 cm., a height of about 22 cm., a usable capacity of approximately 5 liters, and a flat bottom with a drainage hole in the center. They are of sufficient size to grow many species of plants to maturity if care is taken to provide adequate amounts of nutrient solution, particularly during periods when transpiration rates are high. Three oat plants reaching to a height of about 1½ m. have been grown to maturity and single tomato plants with stems exceeding 4 m. in length with yields of more than 14 kgm. of fruit have been grown in pots of this size. When the pot is in use the drainage hole is covered with a watch glass, convex side up. A short piece of glass tubing with an expanded top or a single-hole rubber stopper fitted with a short piece of glass tubing is inserted in the drainage opening if necessary to direct the escaping solution to the waste pan beneath.

Cylindrical crocks (54) of a special design and of a larger capacity than the pot shown in figure 1 but made of the same high-grade ceramic material have also been satisfactory. They are approximately 26.5 cm. in height and 26.5 cm. in outside diameter, have a sloping inside bottom with a hole on the side 32 mm. in diameter, and have a usable capacity of about 7.5 liters. The larger volume and greater exposed surface area when compared with the small pots are of advantage when several plants are grown in a culture.

#### SAND

White quartz sand of a high degree of purity is desirable for experimental work in sand culture and is almost indispensable when the highest degree of nutrient control is to be exercised. Yellowish white silica sand can be used if the traces of impurities such as iron do not interfere with the experimental treatment. The use of mineral particles other than silica and the incorporation in silica sand of mineral particles either difficultly soluble (15) or with a high capacity for nutrient-ion absorption (2, 30, 31) extend the usefulness of the sand culture method in certain types of investigation, but white quartz sand commonly comprises the sole solid component of the substrate. The particle size to be used depends on the height of the sand column in the culture vessel and on the manner of application of the nutrient solution. The important considerations are that there be both adequate aeration for the growth of good root systems and adequate retention of nutrient solution to prevent a possible deficiency of either water or nutrients during periods of rapid water and ion absorption. Because of the relations among particle size, pore spaces, and water-retaining capacity these conditions are fulfilled when the sand particles used in shallow vessels are somewhat larger than those used in tall culture vessels.

When nutrient solution is supplied continuously or very frequently, coarse sand is satisfactory and provides excellent aeration for roots. But when nutrient solution is applied less frequently, sand of somewhat smaller particle size may be necessary to provide an adequate reserve of both water and nutrients in the culture during the period between applications. The use of extremely fine sand is

to be avoided, however, because of the relatively poor aeration afforded. The effects on plant growth of different degrees of aeration (3) and of different moisture contents of the sand (51, 52) have been reported:

Sand closely graded with respect to particle size is desirable for standardization of the cultural factors. Where such sand is unavailable, less closely graded sand can be satisfactorily used, provided it does not contain a large proportion of very small sized particles. The distribution of particle sizes of sand approved for highway concrete construction in this state has been generally suitable for sand cultures with continuous solution renewal. The particle size distribution of the sand used in the culture shown in figure 1 is given in table 1. The particle size distribution of two slightly differing but satisfactory lots of sand has been previously reported (48).

#### NUTRIENT-SOLUTION RESERVOIR

Nutrient-solution reservoirs may take many forms, depending on the manner of nutrient supply (4, 14, 40, 42, 47, 52, 55). The reservoir illustrated (fig. 1C) is a round, 2-quart clear glass, Ball brand canning jar of the glass top and wire

TABLE 1  
*Percentages of different sized particles of white quartz sand*

PARTICLE SIZE	PERCENTAGE OF TOTAL WEIGHT
Larger than 3 mm.	0.3
2-3 mm.	4.8
1-2 mm.	29.6
0.5-1.0 mm.	51.1
0.25-0.5 mm.	7.7
Smaller than 0.25 mm.	6.5

bale type. The inside surfaces of the necks of these jars are smooth and relatively easy to clean. The wire bale is removed, and a notch is ground in the rim of the jar to accommodate the glass capillary delivery tube. The notch is cut to a depth slightly less than the outside diameter of the delivery tube so that the weight of the jar when inverted will hold the tube in any given position after initial adjustment to provide a desired rate of flow. The notch can be readily ground with a narrow, fine grade, power-driven carborundum wheel or with the edge of a carborundum scythe whetstone if the operation is carried out under a stream of water. Similar jars of the same brand having extra large sized mouths are easier to clean than those with standard sized mouths but are less generally available.

The glass dish (fig. 1D) in which the jar stands is known commercially as a "nappy." It has a top outside diameter of 10 cm. and a height of 3.8 cm. With a large mouthed jar, a nappy 12.7 cm. in diameter and 4.4 cm. in height is used.

#### NUTRIENT-SOLUTION DELIVERY TUBE

Capillary glass tubing is very satisfactory for delivering and controlling the flow of nutrient solution to individual sand cultures and is simpler and more

dependable than stopcocks or pinch clamps and rubber tubing. The delivery tube (fig. 1E) is prepared by bending in an S-shape one end of a piece of glass capillary tubing 25 cm. in length, with an outside diameter of 5 to 6 mm. and a bore of about 0.8 mm. This tube delivers nutrient solution at the rate of almost 2 liters a day; a tube with a bore of 0.5 to 0.6 mm. has about half this delivery rate. Because the capillary delivery tube is in reality a siphon, the rate of flow can be regulated over a wide range by adjusting the hydrostatic head between the solution level in the nappie and the lower end of the tube. If the S-shaped end is correctly fashioned this head can be varied from 0 to about 14 cm. by increasing the angle of the tube with the horizontal. In practice the jar is filled with solution, the nappie is placed over the mouth, and both are inverted and placed on the reservoir support. The S-shaped end of the delivery tube is placed in the notch under the rim of the jar and the siphoning action started. The action of the reservoir is that of the Mariotte bottle. The level of the solution in the nappie and the delivery rate of the solution remain fairly constant regardless of the amount of solution in the jar. Periodical cleaning with sulfuric acid-chromate solution prevents clogging of the tube.

#### PLANT, CULTURE VESSEL, AND RESERVOIR SUPPORTS; WASTE PAN

Several types of plant supports have proved very useful. The first consists of a hardwood dowel inserted in a glass tube one end of which is drawn out in a flame to a slender taper and sealed. The glass tube is imbedded in the sand so that the top extends sufficiently above the level of the sand in the pot to prevent nutrient solution from coming in contact with the wooden dowel. Standard sized round hardwood dowels ranging in diameter from 6 to 12 mm. are used, the diameter depending on the size of the plant.

The plant support (H) shown in figure 1 consists of galvanized iron pipe with a bore of 7 mm. and 1.2 m. long fastened to the wooden culture vessel support (G) with lock nuts and washers. The lower part of the plant support is curved so that the upper vertical portion is over approximately the center of the pot. By means of a threaded coupling an additional section of pipe can be attached to the top of the lower section to provide a plant support about 2.4 m. high. This support is entirely outside the pot, and thus contamination of the culture is avoided.

Several tall plants such as tillering grain plants grown in a single culture can be supported by rings of number 9 galvanized iron wire fastened at different heights to the dowel or pipe supports previously described. Soft white heavy cotton string known as "butcher's cord" is excellent for securing plants to upright supports.

The pot support (fig. 1G) is 29.6 cm. square and 12.3 cm. high and is constructed of cypress wood 2.0 cm. thick purchased as "1 by 12" lumber. A round hole 9 cm. in diameter and about 1.6 cm. in front of the center of the top of this support allows the excess solution to escape from the pot to the pan below and locates the pot so that with the plant placed in the center of the pot the stem can be easily fastened in a vertical position to the plant support. This wooden

pot support can be used with 2- or 3-gallon crocks as well as with pots of the size shown in figure 1. The reservoir support (fig. 1F) is of cypress wood 2.0 cm. thick with the exception of the round fir upright 4.5 cm. in diameter. The base is 14.3 cm. square; the circular top is 10.2 cm. in diameter. The over-all height of the support is 42.8 cm. and such that the upper surface of the top is about 9 cm. above the rim of the culture vessel. The waste pan (fig. 1I) is of heavily enameled steel, 26.6 cm. in diameter and 9 cm. high, and has a capacity of 3 liters. It is dark in color to discourage the growth of algae.

#### PREPARATION OF EQUIPMENT FOR USE

Culture vessels and glassware are washed with tap water and if necessary with acid cleaning solution or with an alkaline cleaning compound and rinsed with distilled water. Sand is washed by putting a sufficient amount in an 8- to 11-liter crock to fill it one-half to two-thirds full and then by running into it a full stream of water from the open end of a garden hose kept slightly off the bottom of the crock. Washing is continued with a swirling motion and with frequent decanting of supernatant water until visible impurities are removed and the wash water runs clear. In order to reduce further the content of impurities such as organic matter or inorganic substances not readily removed with distilled water, the sand is flushed several times with a 3 per cent solution of hydrochloric acid and then with a sodium hydroxide solution of approximately the same concentration, each treatment being followed in turn by thorough flushings with distilled water. The acid and alkali treatment is conveniently carried out in 19- or 38-liter, round-bottomed, glazed crocks with bottom drainage holes. Before placing the sand in the pots it is well to test the pH value of the flushing waste following the alkali treatment and to flush the sand if necessary with a very dilute acid solution or with a buffered nutrient solution until the pH of the final waste solution reaches a satisfactory value. After the clean sand is put in the culture vessels, it is flooded with distilled water or with the nutrient solution which is to be used in the experiment and settled by jarring the pot several times to eliminate large air pockets.

#### PREPARATION OF PLANT MATERIAL

Seeds used for the production of plants for experimental purposes are selected carefully for uniformity of size, shape, and maturity. Seed-coat color is often a valuable index of maturity. If seed stock is of a high degree of genetic purity and particularly with species where the stored reserve of the seed is large and where the length of the experimental period from seed germination to harvest is of short duration, additional selection by weighing individual seeds is likely to increase further the degree of uniformity of the resulting seedlings.

Seeds may be germinated in sand by uniformly spacing them in shallow furrows and covering them with sand to a depth depending on the size and kind of seed. If planted approximately 6 mm. deep, tomato seeds produce satisfactory seedlings. Seeds may be planted directly in sand in the culture vessels if the seedlings are difficult to transplant, but it is usually more satisfactory to

plant them in separate germination containers and to transplant the seedlings after rigid selection for uniformity of both top and root system.

Germination boxes 40.5 cm. long, 14.3 cm. high, and 18.9 cm. wide, of the type previously illustrated (55), have been found to be of convenient size and are large enough in which to start 75 to 100 seedlings in three rows. These boxes are conveniently made of standard size 1- by 6-inch cypress lumber 2.0 cm. thick. Drainage is provided by leaving about 2 mm. cracks between the edges of the bottom board and the sides of the box and covering the cracks with fiber glass to retain the sand. The box is filled to within about 2.5 cm. from the top with washed sand, which is then flooded with a suitable germinating nutrient solution and jarred slightly to eliminate large air pockets. Seedlings may be protected against loss by damping off diseases by flooding the sand with sufficient hot water so that the temperature of the sand mass remains at 80°C. or higher for at least 20 minutes. After the seed has been planted the box is covered with a sheet of glass and held at a suitable germinating temperature. After the seedlings appear above the surface of the sand the glass cover is raised slightly to provide for a small amount of air circulation. This helps to prevent development of excessively soft plant tissues. Usually the temperature suitable for seedling growth is somewhat lower than that desirable for seed germination, but extremes of temperature are to be avoided. Care should be taken as long as the glass cover is in place to locate the box of seedlings where direct sunlight will not cause the temperature to become undesirably high. The range of temperatures to be maintained is determined largely by the condition of the seedlings. The appearance of low-light symptoms and spindling growth is usually a signal to lower the temperature slightly if it is not practical to provide additional light. Spindling or very soft seedlings usually recover slowly and nonuniformly after being transplanted into the cultures and, therefore, are undesirable.

Seedlings may be transplanted into the sand in the culture pots as soon as they have reached a stage when there is a reasonably good basis for judgment in the selection for uniformity. This occurs before there is extensive lateral root development and with dicotyledonous plants is often about the time when the first true leaves start to develop. The seedlings may be withdrawn from the germinating box with a minimum amount of injury by flooding the sand with water and by assisting the removal with a porcelain spatula if necessary. Transplanting is done by carefully placing the root system in a hole in the sand large enough to accommodate the roots without crowding and then, while suspending the seedling in the desired position, flooding the sand with nutrient solution. This allows the sand to flow about and make close contact with the roots. It is generally of advantage to transplant the seedlings slightly deeper than they were in the germination box.

If plants are to be produced by asexual propagation, cuttings may be rooted in white quartz sand. Propagating chambers such as are used to root cuttings in the greenhouse are excellent for this purpose where many cuttings are to be rooted. For small numbers of cuttings, glazed crocks holding 8 to 11 liters (57) and about two-thirds full of washed sand have proved satisfactory as rooting

containers for a number of species of plants. During the rooting period the sand is kept moistened with tap water, if this does not interfere with the experiment, or with a nutrient solution of about one-quarter or less of the usual salt concentration and containing very little nitrogen or lacking nitrogen entirely. Free bottom drainage is necessary to provide adequate aeration of the sand for good root development. The crocks are covered almost completely with a sheet of glass to maintain high humidity in the air over the sand and are located in the greenhouse so that they receive skylight but not the direct rays of the sun.

#### CHOICE OF NUTRIENT SOLUTION

There is no one nutrient solution which is superior to all others under widely differing environmental conditions. The range of equally favorable nutrient salt ratios is fairly broad (20). Although a given solution might be superior to others at a given time, constantly changing conditions of both plant and environment bring about constantly changing nutrient requirements. Obviously the species of plant to be grown, the nature of the experiment, and the seasonal conditions affect the choice of the nutrient solution. Where the effect of an environmental limiting factor such as light intensity, length of photoperiod, or temperature is to be studied, a supply of all essential nutrients is properly provided which will be in excess of the probable nutrient requirements at all times during the experimental period. When the effect of a limited supply of one essential nutrient is to be studied, other nutrients are provided in some degree of excess of the probable maximum nutrient requirements during the same period. Unnecessarily high concentrations of nutrient ions are to be avoided because of undesirable antagonistic, toxic, osmotic, or specific effects. An unnecessarily high concentration of nitrogen, for example, may greatly affect the character of vegetative or reproductive responses to be induced by the limiting factor under study.

A good nutrient solution has a certain degree of physiological balance (38), that is, a balance between those ions which are antagonistic or competitive with respect to ion absorption or utilization as, for example, calcium and potassium, and this requirement also imposes a restriction upon the composition of a nutrient solution. The allowable range in ion proportions is not narrow, however, and ordinarily becomes critical only when there is an incipient deficiency of an essential element.

A complete nutrient solution is ordinarily prepared to supply relatively large amounts of nitrogen and potassium, intermediate amounts of calcium, magnesium, sulfur, and phosphorus, and small amounts of elements used in traces such as iron, manganese, boron, and zinc. Other essential trace elements, including copper and molybdenum, are usually present as impurities in the salts or water in sufficient amounts so as not to limit growth but may be added as a precaution against possible deficiency. Nitrogen, phosphorus, and sulfur may be supplied as the nitrate, phosphate, and sulfate respectively of potassium, calcium, or magnesium making six possible combinations of salts which can supply these elements without duplication of any ion (34, 35).

The best way to determine the composition of a nutrient solution suitable for a given plant species under a definite set of environmental conditions is to grow plants with a series of solutions in which the relative proportions of salts are systematically varied and to select those solutions which allow desirable growth responses. This method has been used with three- and four-salt component systems (29, 50, 61). When only three or four salts are used, solutions formulated according to a triangle or pyramidal system have the valuable advantages of simplicity and of equality of osmotic pressure but have the disadvantage that any two solutions differ from each other with respect to the ions of at least two different essential elements. This complicates the interpretation of experimental results of experiments designed to test the effect of a variation of a single nutrient element.

There is probably no completely satisfactory solution to the problem of testing the effect of variations of but a single ion. But by using a combination of a large number of salts in which each essential nutrient ion is common to more than one salt, this disadvantage can be partly eliminated (12, 18, 64).

In order to study the effect of a deficiency or an excess of a single element, nutrient solutions are used in which variations of the ion containing the element to be studied are paired with variations of one or more of those ions of opposite charge which have the least significance in the experiment. For example, in many experiments not involving these ions primarily, the magnesium and sulfate ions may be varied throughout a considerable range in concentration above a minimum critical level which induces characteristic deficiency symptoms in the plant with less apparent effect on metabolism than would accompany similar variations of other essential ions. Similarly the concentrations of sodium and chloride ions can be varied over a wide range without apparent serious effect on the growth of some, though perhaps not of other, plant species. It is, of course, realized that it is not possible to vary the concentration of any single nutrient ion without affecting the pH value of the solution, and conversely the pH value of a given nutrient solution cannot be changed without affecting the concentration of at least one other ion comprising the nutrient solution.

Nutrient solutions for use in sand cultures are usually most satisfactory if their osmotic pressure values are not more than about one atmosphere. Plants absorb water with difficulty from nutrient solutions of high osmotic pressure values (62) and severe water stresses are set up in the plant which may result in such diseases as blossom-end rot of tomatoes (48). Nutrient solutions of approximately one-half atmosphere have been used successfully for a number of years and provide adequate nutrients for the growth of excellent plants when applied at the rate of about 1,000 to 2,000 ml. per culture daily. There is no lower limit for osmotic pressure as such, but throughout the range of relatively low osmotic pressure values where the salt concentrations are necessarily low there is an inverse relationship between the nutrient concentration and the volume of solution that must be applied to provide an adequate supply of nutrients (1, 11, 19, 27, 44, 48). Often this relationship approaches proportionality and must be taken into consideration in the determination of the concentration of the nutrient solution to be used.

Nutrient solutions are generally most satisfactory if their pH values lie between 4.8 and 6.0, and with some species up to 6.2 or 6.4, although it has been shown that a considerably wider range of pH values can be used if concentrations of available nutrient ions are carefully controlled (8, 9). Solutions containing monobasic potassium phosphate as one of the constituents have pH values that vary from approximately 4.8 to 5.0. When the monobasic phosphates of calcium or magnesium are used as sole sources of phosphorus, the pH values of the solutions are usually below 4.0. They may be adjusted to 5.0 or higher with appropriate amounts of the hydroxides or dibasic phosphates of one or more of the essential or nontoxic bases. They may be lowered by the addition of appropriate amounts of nitric, sulfuric, phosphoric, or hydrochloric acid. The pH values of nutrient solutions often change markedly from their original values in passing through the cultures as a result of unequal rates of absorption of nutrient cations and anions. When ammonium sulfate is used to provide the sole source of nitrogen, pH values may in some cases decrease below 4.0 (45), and the roots may be severely injured. When calcium nitrate is used to provide the sole source of nitrogen, pH values may rise to a point where the concentration of soluble iron becomes low enough to cause iron-deficiency chlorosis. The use of both forms of nitrogen in a nutrient solution tends to reduce the extent of such fluctuations in pH values (63, 65). Ammonium can be used satisfactorily as the sole source of nitrogen if the pH values of the solution in the culture are kept from falling too low (28, 41, 60). During periods of low light intensity, however, ammonium nitrogen should be used only in relatively low concentrations or eliminated from the solution to prevent injury to the plant. The rapid absorption rates by plant roots of the ions of ammonium, potassium, and nitrate as compared with those of other nutrients makes their presence important in a nutrient solution in helping to balance the rates of absorption of cations and anions. Although much can be done by reducing the extent of pH changes, if it is desired to approach a constancy in pH values, the necessity of frequent modification of the nutrient solution must be anticipated.

Though, as already pointed out, it is not possible to say what composition of a nutrient solution will be the best under widely differing conditions, the following ranges in molar concentration can be used for good growth of a number of plant species in sand culture: nitrate 0.005 to 0.010; phosphate 0.00025 to 0.002; sulfate 0.001 to 0.010; potassium 0.002 to 0.010; calcium 0.002 to 0.005; and magnesium 0.001 to 0.010. For the production of more or less marked deficiency symptoms of any of these inorganic nutrients the concentrations must be reduced to approximately one-tenth or less of the lower values of the ranges stated.

Table 2 gives the composition of several solutions that have been successfully used with a number of different species of plants. A more complete discussion of nutrient requirements and nutrient solutions is given in a recent publication (36). Formulas 1 and 2 contain ammonium nitrogen, formulas 3 and 4 include two different concentrations of nitrate nitrogen. In addition to the salts listed in table 2 certain trace elements, also termed "micronutrient elements" (25) or "minor elements" (13), must be supplied. The following concentrations of trace elements are satisfactory for the growth of many plant species: boron,

manganese, and zinc 0.25 p.p.m. each, and iron 0.5 p.p.m. Boron is conveniently used in the form of boric acid, and manganese, zinc, and iron are used as sulfates, although organic compounds of some of these elements are useful over a wider range of pH values than are the inorganic compounds (26). When distilled water and nutrient salts are very carefully purified, significant increases in growth have been obtained with the addition of other trace elements, and two of these, namely, copper and molybdenum, have been shown to be especially important for optimum growth (5, 6, 7, 23). Concentrations of 0.02 p.p.m. of copper as the sulfate and of 0.01 p.p.m. molybdenum as molybdic acid apparently are satisfactory for a number of plant species, but higher concentrations may be necessary for other species (23, 25).

#### PREPARATION OF NUTRIENT SOLUTIONS

Nutrient solutions used for experimental work in plant nutrition are ordinarily prepared from inorganic salts of reagent or analytical grade. If it is suspected that salts contain as impurities trace elements in amounts that would interfere

TABLE 2

*Composition of several nutrient solutions used for growing plants in sand culture\**

NO.	INVESTIGATOR	MOLAR CONCENTRATION					
		KH <sub>2</sub> PO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	KNO <sub>3</sub>	K <sub>2</sub> SO <sub>4</sub>
1	Arnon (5)		.0010		.0040	.0060	
2	Shive and Robbins (55)	.0023		.0007	.0045		.0023
3	Author	.0010			.0050	.0020	.0020
4	Author	.0010			.0050	.0040	.0020

\* Refer to discussion concerning concentration of trace elements to be used.

with the problem at hand, samples are tested either chemically or biologically. Samples of several lots can sometimes be obtained from chemical supply houses and that lot purchased which is most satisfactory when judged on the basis of the tests. For example, the approximate boron contents of nitrates for which the quinalizarin method for boron is not suitable have been determined by using the salts one at a time as a component of a nutrient solution with other salts the boron contents of which are known and comparing the response of plants with those of plants supplied with definite concentrations of boron. Purification of salts by recrystallization or by special methods (6, 58, 59) is advisable when the effects of a deficiency of trace elements such as copper, zinc, or molybdenum are to be studied.

Distilled water from a Barnstead still has been used where a study of the elements used in major quantities and also certain of the trace elements is involved. For work with certain other trace elements like copper and zinc, water can be redistilled from Pyrex glass or otherwise purified (6, 59). Purification by ion exchange appears to be a promising means of obtaining water of satis-

factory purity for the investigation of certain trace elements (32). Tap water, although satisfactory for commercial plant production, is unsatisfactory for experimental work in plant nutrition when salts of analytical reagent grade purity are required.

Nutrient solutions are readily prepared from nutrient salt stock solutions. Stock solutions of salts used in large quantities by plants are convenient if prepared to be one-half volume molecular, except that when the solubility of the salt does not permit this concentration, lower concentrations are used. Stock solutions of the trace elements are convenient to use if prepared to contain the element in question in terms of milligrams per milliliter. One milliliter of such stock solution used in the preparation of 1 liter of nutrient solution then represents a concentration value of the trace element in terms of parts per million equivalent to the number of milligrams per milliliter of the stock solution.

It is advisable to keep stock solutions out of bright light or to store them in brown bottles to retard changes in composition and the growth of algae. Iron salts remain in the ferrous condition longer than otherwise if about 0.05 ml. of concentrated sulfuric acid is added per liter of stock solution.

To prepare the nutrient solution for use, the necessary amounts of the stock solutions are measured in graduate cylinders, burettes, or pipettes depending upon the degree of accuracy required. The measured amounts are added to calibrated glass containers about half full of distilled water. Volumetric flasks and glass bottles with the calibration mark at the neck are convenient for this purpose. A large glass rod has been found useful for thoroughly mixing the nutrient solution after making to volume.

Because of the variation in moisture content of the salts, half molar stock solutions of certain highly deliquescent salts such as calcium nitrate are prepared by adjusting the specific gravity to the proper value by dilution with water. The specific gravity readings are made with a narrow-range hydrometer or with a pycnometer. Exact values of the nutrient-ion composition of a solution when desired for quantitative studies involving ion absorption are determined by chemical analysis.

#### APPLICATION OF NUTRIENT SOLUTION

Nutrient solution may be applied to sand cultures in several ways, but no way has been found to produce better plants than that of application to the surface by continuous solution renewal (53, 55). This method consists of allowing unused nutrient solution to drip continuously on the surface of the sand, the excess solution escaping from the bottom of the culture vessel, and at regular intervals flushing the sand with unused nutrient solution. Solution is easily applied to the surface of the sand by means of the capillary delivery tube previously described. The rate of flow to be used depends upon the number of plants per culture, the size of the plants, the nature of the experiment, and the environmental conditions. High delivery rates are used when the requirement for water and nutrients is high. While no fixed directions can be given, a rate of 1 liter per culture per 24 hours is satisfactory for small plants, and 2 liters or more a day is

often used for large plants. In general, the rates used should provide those essential nutrients which are not being intentionally limited and water in considerable excess of the requirements of the plant.

One rough gauge of the proper rate of solution application is the relative amount of the solution escaping from the culture. Consistent with practical considerations, the more nearly the volume of the escaping solution approaches that supplied, the better, but if this amount is less than approximately half of the volume supplied, changes in the solution which have occurred while passing through the culture may have been undesirably great. Changes in the specific electrical conductivity of the escaping solution as compared with that of the original solution indicate the magnitude of the nutrient-ion changes. The conductivity determinations are convenient to make and are useful in determining a suitable rate of solution application. Another guide in determining the rate of solution application is the extent of the change in pH values between that of the original and that of the escaping solution. As pointed out previously the composition of the solution can be adjusted as a means of preventing great changes in pH value, and this method is to be preferred where possible. Otherwise, for example, when ammonium is the sole form of nitrogen present it may be necessary to use much higher rates of solution application to prevent extreme changes in pH value of the solution as it passes through the culture than when both forms of nitrogen are present.

Where the effects of the deficiency of a nutrient element are to be studied, it is better to reduce the concentration of the element in question than to limit severely the volume of the nutrient solution applied. The latter method is unsatisfactory because of the multiple effects on metabolism accompanying a limited water supply. Many very puzzling symptoms of malnutrition of plants grown in sand culture occur as a result of the use of insufficient nutrient solution. Actually these symptoms may be associated with nutrient deficiencies or with undesirable changes in the osmotic pressure, pH value, and nutrient-ion proportion of the solution. There is probably no single factor more important in growing plants in sand cultures than that of supplying adequate amounts of nutrient solution, and it is difficult to attach too much emphasis to this consideration.

Considerable attention has been given to the maintenance of constant rates of nutrient solution application from hour to hour and from day to day. It is desirable that the rates of solution application be under the best possible control so that cultural conditions may be standardized and easily duplicated if desired. But with fluctuating greenhouse temperature it is obviously virtually impossible to maintain constant rates of solution application when delivery rates are controlled in part by the resistance to the flow through a capillary tube unless the position of the tube is changed whenever the temperature changes. The resistance to flow through capillary tubes of the size used is partly dependent upon the viscosity of the water. Changes in temperature which are common in the greenhouse appreciably affect the viscosity of water and, therefore, in turn affect the rate of solution delivery. If all capillary delivery tubes are of uniform

bore, however, such changes in rate of flow will be similar. Furthermore, it is not necessarily undesirable that the rate of flow automatically increases slightly as the temperature rises, because temperature rises ordinarily coincide with increases in light intensity and are accompanied by increases in rates of transpiration and of nutrient-ion absorption. The slowest rate of flow through capillary delivery tubes occurs usually at night during the period of low temperatures when rates of transpiration and water absorption are ordinarily at the minimum point for the 24-hour period. The absorption of nutrients by plants can be followed more or less closely in sand culture by maintaining a quantitative balance sheet of the nutrients added to, escaping from, and remaining in the culture (16, 30), although this cannot be done quite so easily as when plants are grown in solution cultures.

Supplementary to the continuous solution renewal procedure it is advisable to flush each sand culture at regular intervals with the nutrient solution in use. This flushing renews the solution in the upper portions of the sand at some distance from the point where the solution drips and prevents possible salt accumulations on the surface of the sand. A volume of 600 ml. is ordinarily sufficient for this purpose with a pot of the type illustrated in figure 1 when the sand is close to the saturation point, but in any case the amount to be used should be considerably in excess of that required to completely saturate the sand. Daily flushings are advisable as a general rule but they may be made more or less frequently depending on whether plants are very large or very small and whether the transpiration and evaporation rates are very high or very low.

Nutrient solution can be supplied to plants grown in sand cultures by other means than by continuous solution renewal, although special precautions are necessary to control the supply of nutrients, water, and oxygen available to the roots. Nutrient solution can be applied intermittently in sufficient amounts to constitute a thorough flushing. This can be done by hand (40) or by some mechanical system (14, 17). The frequency of such application may vary from daily to several times daily but should be carried out so that the supply of water and of any nutrient not being intentionally limited does not become a limiting factor for growth.

Nutrient solution can be supplied to sand cultures by other means than that of surface application. Nutrient solution can be supplied by capillary absorption either in a closed system under tension (30) using the principle of the porous clay absorbing surface (33, 46) or by porous clay wicks (42). Pots may also be placed in enamel pans containing a supply of nutrient solution (40), the sand in the culture acting as a capillary medium, but this method is not satisfactory with small pots. Supplying nutrient solution solely by methods of capillarity has the advantage of automatically maintaining a more or less uniform water tension in the culture, provided the water-absorbing capacity is great enough to provide for maximum transpirational requirements, but it has the disadvantage of a lack of good control of nutrient-ion concentration. This disadvantage becomes increasingly serious as the length of the experimental periods increase and plants increase in size.

Excellent plants can be grown by the method of subirrigation in which the sand

mass is flooded from below, provided that the sand volume in the cultures is large enough to afford a considerable reserve of solution between subirrigations and provided further that these operations are carried out frequently and that coarse enough sand is used to ensure rapid and uniform drainage. Otherwise, this method presents disadvantages similar to those of intermittent surface application of nutrient solution and, in addition, fails to provide for satisfactory displacement of used solution. In subirrigation techniques the surplus solution is withdrawn either by suction (37, 50) or by gravity (42). The displacement of used solution with unused solution is not so complete in a single subirrigation of this type as in a flushing with an equal amount of solution applied to the surface.

Subirrigation procedures are usually used instead of surface application to save time in daily care of plants. When large numbers of plants are grown with a single solution in a single large container, this saving of time is considerable and the method is thoroughly practical as, for example, in greenhouse benches. Any time gained by using subirrigation is due largely to the reuse of the nutrient solution. This means that a solution of changing composition is being introduced into the culture unless after each contact with the plant roots the solution is tested and restored to its original composition, a task in itself of no small magnitude where many different cultural treatments are involved. The extent of nutrient changes can be kept relatively small by using large volumes of solution (14). This is obviously important where this method is used. Where space is limited, however, large solution reservoirs are cumbersome.

In the choice of technique of solution application a number of factors must be considered, but it is important that the nutrient supply be subject to the highest practical degree of control and that the conditions of nutrient supply be definitely known as far as possible and be capable of duplication.

#### ATMOSPHERIC CONDITIONS

Although not confined to the growth of the plants in sand culture the success of a plant nutrition experiment depends largely on the degree of control of light, temperature, humidity, and other environmental conditions.

Uniformity of light conditions for all comparative cultures is particularly important. A light meter may be used to determine light intensity. Pots may be rotated regularly to help compensate for minor differences in exposure. During winter particularly, greenhouse glass should be kept clean. The presence of dust or dirt on glass, which might easily pass unnoticed, has been found to cause decreases of 10 or 15 per cent in the intensity of light that would otherwise be available in the greenhouse. During winter, differences in light intensity of this magnitude can markedly alter the course of plant metabolism.

Extremes of temperature are to be avoided. As a general rule, optimum desirable temperatures vary directly, although not necessarily proportionately, with the light intensity and the length of day and also depend on the species of plant. It is important that the night temperature be limited to a definite maximum during the winter season in order to conserve the carbohydrate supply of the plant. Day temperatures are ordinarily satisfactory for many species if main-

tained from about 6 to 11°C. higher than the night temperature, depending on the total daily light energy. For tomato, for example, the desirable night temperature during the winter season in New Brunswick, New Jersey, lies between about 13 and 16°C. and the day temperature between about 19 and 28°C. During the summer both day and night temperatures are satisfactory if somewhat higher than the respective winter temperature ranges, but temperatures in the greenhouse exceeding about 32°C. are as a rule undesirable at any season of the year. Control of temperature maximum during the summer is sufficiently important to justify the reduction of light intensity by suitable shading of greenhouse glass if necessary.

It is important to control the relative humidity, which for best results should generally be maintained above 50 per cent. For certain species like the rose and the gardenia, considerably higher relative humidities are desirable, but values above 90 per cent ordinarily are unnecessary or are to be avoided. Toxic gases like carbon monoxide, ethylene, or artificial illuminating gas often cause puzzling symptoms of injury and should be excluded from the greenhouse. The importance of the various factors of the environment particularly as they affect the nitrogen metabolism (40) of plants is not to be overlooked.

This paper has laid some emphasis on techniques used in growing plants in sand culture. Techniques serve as guides and are valuable *per se*, but they possess the most value when all the environmental conditions are completely controlled. A particular predetermined technique is not necessarily to be followed in all its details from the beginning to the end of an experiment to the possible detriment of the experimental results. The primary desideratum is that the response of the plant to a given set of environmental conditions yield the desired answer to the original question. Insofar as the environmental conditions cannot be predicted in advance or controlled, so must such unavoidable changes in the environmental factors be compensated for, if necessary by appropriate modifications in techniques of culture, as occasions arise and as the appearance and behavior of the plants dictate.

#### REFERENCES

- (1) AHLGREEN, G. H. 1941 Some effects of volume rate of solution supply and of potassium concentrations on the growth of white clover. *Soil Sci.* 52: 229-235.
- (2) ALRECHT, W. A. 1932 Calcium and hydrogen ion concentration in the growth and nodulation of soybeans. *Jour. Amer. Soc. Agron.* 24: 793-806.
- (3) ALLISON, R. V. 1922 The relation of aeration to the development of the soybean plant in artificial culture. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1921: 338-344.
- (4) ALLISON, R. V., AND SHIVE, J. W. 1923 Studies on the relation of aeration and continuous renewal of nutrient solution to the growth of soybeans in artificial culture. *Amer. Jour. Bot.* 10: 554-566.
- (5) ARNON, D. I. 1938 Microelements in culture-solution experiments with higher plants. *Amer. Jour. Bot.* 25: 322-325.
- (6) ARNON, D. I., AND STOUT, P. R. 1939 The essentiality of certain elements in minute quantity for plants with special reference to copper. *Plant Physiol.* 14: 371-375.
- (7) ARNON, D. I., AND STOUT, P. R. 1939 Molybdenum as an essential element for higher plants. *Plant Physiol.* 14: 599-602.

- (8) ARNON, D. I., FRATZKE, W. E., AND JOHNSON, C. M. 1942 Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. *Plant Physiol.* 17: 513-524.
- (9) ARNON, D. I., AND JOHNSON, C. M. 1942 Influence of hydrogen ion concentration on the growth of higher plants under controlled conditions. *Plant Physiol.* 17: 525-539.
- (10) ARNON, D. I. AND HOAGLAND, D. R. 1944 The investigation of plant nutrition by artificial methods. *Biol. Rev. Cambridge Phil. Soc.* 19: 55-67.
- (11) BARTHOLOMEW, R. P., AND JANSSEN, G. 1929 Relation between concentration of potassium in culture solutions and optimum plant growth. *Soil Sci.* 27: 189-203.
- (12) BECHENBACH, J. R., WADLEIGH, C. H., AND SHIVE, J. W. 1936 Nutrition studies with corn: I. Statistical interpretation of the nutrient ion effect upon growth in artificial culture. *Soil Sci.* 41: 469-489.
- (13) BRENCLEY, W. E. 1936 The essential nature of certain minor elements for plant nutrition. *Bot. Rev.* 2: 173-196.
- (14) CHAPMAN, H. D., AND LIEBIG, G. F., JR. 1938 Adaptation and use of automatically operated sand-culture equipment. *Jour. Agr. Res.* 56: 73-80.
- (15) CHAPMAN, H. D. 1939 Absorption of iron from finely ground magnetite by citrus seedlings. *Soil Sci.* 48: 309-315.
- (16) DAVIDSON, O. W., AND SHIVE, J. W. 1934 The influence of the hydrogen-ion concentration of the culture solution upon the absorption and assimilation of nitrate and ammonium nitrogen by peach trees grown in sand cultures. *Soil Sci.* 37: 357-385.
- (17) GAUCH, G., AND WADLEIGH, C. H. 1943 A new type of intermittently irrigated sand culture equipment. *Plant Physiol.* 18: 543-547.
- (18) HAMNER, K. C., LYON, C. B., AND HAMNER, C. L. 1942 Effect of mineral nutrition on ascorbic-acid content of the tomato. *Bot. Gaz.* 103: 586-616.
- (19) HOAGLAND, D. R. 1919 Notes on the technique of solution culture experiments with plants. *Science (n.s.)* 49: 360-362.
- (20) HOAGLAND, D. R. 1920 Optimum nutrient solutions for plants. *Science (n.s.)* 52: 562-564.
- (21) HOAGLAND, D. R. 1937 Some aspects of the salt nutrition of higher plants. *Bot. Rev.* 3: 307-334.
- (22) HOAGLAND, D. R., AND ARNON, D. I. 1938 The water culture method for growing plants without soil. *Calif. Agr. Exp. Sta. Cir.* 347.
- (23) HOAGLAND, D. R. 1941 Water culture experiments on molybdenum and copper deficiencies of fruit trees. *Proc. Amer. Soc. Hort. Sci.* 38: 8-12.
- (24) HOAGLAND, D. R., AND ARNON, D. I. 1941 Physiological aspects of availability of nutrients for plants growth. *Soil Sci.* 51: 431-444.
- (25) HOAGLAND, D. R. 1944 Lectures on the Inorganic Nutrition of Plants. Chronica Botanica Co., Waltham, Mass.
- (26) HORNER, C. K., BURK, D., AND HOOVER, S. R. 1934 Preparation of humate metals. *Plant Physiol.* 9: 663-669.
- (27) JOHNSTON, E. S. AND HOAGLAND, D. R. 1929 Minimum potassium level required by tomato plants grown in water cultures. *Soil Sci.* 27: 89-109.
- (28) JONES, L. H., AND SHIVE, J. W. 1921 Effect of ammonium sulfate upon plants in nutrient solutions supplied with ferric phosphate and ferrous sulphate as sources of iron. *Jour. Agr. Res.* 21: 701-728.
- (29) JONES, L. H., AND SHIVE, J. W. 1923 Influence of ammonium sulfate on plant growth in nutrient solutions and its effect on hydrogen-ion concentration and iron availability. *Ann. Bot.* 37: 355-377.
- (30) KOLODNEY, L., AND ROBBINS, W. R. 1940 Availability of fixed potassium to plants. *Soil Sci.* 49: 303-313.

- (31) KUNIN, R., AND ROBBINS, W. R. 1944 The relative availability to plants of exchangeable calcium from soil separates of sand, silt, and clay. *Soil Sci.* 57: 137-142.
- (32) LIEBIG, G. F., JR., VANSELLOW, A. P., AND CHAPMAN, H. D. 1943 The suitability of water purified by synthetic ion-exchange resins for the growing of plants in controlled nutrient cultures. *Soil Sci.* 55: 371-376.
- (33) LIVINGSTON, B. E. 1918 Porous clay cones for the auto-irrigation of potted plants. *Plant World* 21: 202-208.
- (34) LIVINGSTON, B. E., AND TOTTINGHAM, W. E. 1918 New three-salt nutrient solution for plant cultures. *Amer. Jour. Bot.* 5: 337-346.
- (35) LIVINGSTON, B. E. (Editor) 1919 A plan for cooperative research on the salt requirements of representative agricultural plants prepared for a special committee of the Division of Biology and Agriculture of the National Research Council. Baltimore.
- (36) LIVINGSTON, B. E. 1942 Mineral requirements of plants as indicated by means of solution cultures. *Amer. Assoc. Adv. Sci. Pub.* 16: pp. 83-111.
- (37) MCCALL, A. G. 1915 A new method for the study of plant nutrients in sand cultures. *Jour. Amer. Soc. Agron.* 7: 249-252.
- (38) MCCALL, A. G. 1916 The physiological balance of nutrient solutions for plants in sand cultures. *Soil Sci.* 2: 207-254.
- (39) MARSH, R. P. 1942 Comparative study of the calcium-boron metabolism of representative dicots and monocots. *Soil Sci.* 53: 75-78.
- (40) NIGHTINGALE, G. T., SCHERMERHORN, L. G., AND ROBBINS, W. R. 1932 Effects of sulphur deficiency on metabolism in tomato. *Plant Physiol.* 7: 565-595.
- (41) NIGHTINGALE, G. T. 1937 The nitrogen nutrition of green plants. *Bot. Rev.* 3: 85-174.
- (42) NOREM, W. L. 1936 Mineral nutrition and seasonal growth of ageratum in sand cultures with auto-irrigation. *Amer. Jour. Bot.* 23: 545-555.
- (43) PADDICK, M. E. 1944 A simple apparatus for sand culture work. *Plant Physiol.* 19: 704-705.
- (44) PARKER, F. W., AND PIERRE, W. H. 1928 Relation between the concentration of mineral elements in a culture medium and the absorption and utilization of those elements by plants. *Soil Sci.* 25: 337-343.
- (45) PRINCE, A. L., JONES, L. H., AND SHIVE, J. W. 1923 Notes on differential ion absorption by plants in relation to reaction changes in nutrient solutions. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1922: 378-383.
- (46) RICHARDS, L. A., AND BLOOD, H. L. 1934 Some improvements in auto-irrigator apparatus. *Jour. Agr. Res.* 49: 115-121.
- (47) ROBBINS, W. R. 1929 The possibilities of sand culture for research and commercial work in horticulture. *Amer. Soc. Hort. Sci.* 25: 368-370.
- (48) ROBBINS, W. R. 1937 Relation of nutrient concentration to growth of the tomato and to the incidence of blossom-end rot of the fruit. *Plant Physiol.* 12: 21-50.
- (49) SHIVE, J. W. 1915 A study of physiological balance in nutrient media. *Physiol. Res.* 1: 327-397.
- (50) SHIVE, J. W. 1918 A comparative study of salt requirements for young and for mature buckwheat plants in sand cultures. *Soil Sci.* 6: 1-32.
- (51) SHIVE, J. W. 1920 Relation of moisture in solid substrate to physiological salt balance for plants and to the relative plant producing value of various salt proportions. *Jour. Agr. Res.* 18: 357-378.
- (52) SHIVE, J. W. 1920 The influence of moisture content of sand cultures upon the physiological salt balance for plants. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1919: 358-363.
- (53) SHIVE, J. W., AND STAHL, A. L. 1927 Constant rates of continuous solution renewal for plants in water cultures. *Bot. Gaz.* 84: 317-323.

- (54) SHIVE, J. W. 1936 The adequacy of the boron and manganese content of natural nitrate of soda to support plant growth in sand culture. N. J. Agr. Exp. Sta. Bul. 603.
- (55) SHIVE, J. W., AND ROBBINS, W. R. 1937 Methods of growing plants in solution and sand cultures. N. J. Agr. Exp. Sta. Bul. 636.
- (56) SHIVE, J. W. 1940 Sketch of the development of the water culture method of growing plants. *Sci. Mo.* 51: 233-240.
- (57) SPENCER, E. L., AND SHIVE, J. W. 1933 The growth of *Rhododendron ponticum* in sand cultures. *Bul. Torrey Bot. Club* 60: 423-439.
- (58) STEINBERG, R. A. 1935 Nutrient solution purification for removal of heavy metals in deficiency investigations with *Aspergillus niger*. *Jour. Agr. Res.* 51: 413-424.
- (59) STOUT, P. R., AND ARNON, D. I. 1939 Experimental methods for the study of the role of copper, manganese, and zinc in the nutrition of higher plants. *Amer. Jour. Bot.* 26: 144-149.
- (60) TIEDJENS, V. A., AND ROBBINS, W. R. 1931 The use of ammonia and nitrate nitrogen by certain crop plants. N. J. Agr. Exp. Sta. Bul. 526.
- (61) TETTINGHAM, W. E. 1914 A quantitative chemical and physiological study of nutrient solutions for plant cultures. *Physiol. Res.* 1: 133-245.
- (62) TRELEASE, S. F. 1917 The relation of the concentration of the nutrient solutions to the growth of young wheat plants in water cultures. *Johns Hopkins Univ. Cir. (n.s.)* 3: 225-227.
- (63) TRELEASE, S. F., AND TRELEASE, H. M. 1935 Changes in hydrogen-ion concentration of culture solutions containing nitrate and ammonium nitrogen. *Amer. Jour. Bot.* 22: 520-542.
- (64) WADLEIGH, C. H. 1939 The influence of varying cation proportions upon the growth of young cotton plants. *Soil Sci.* 48: 109-120.
- (65) WOLKOFF, M. L. 1918 Effect of ammonium sulfate in nutrient solution on the growth of soybeans in sand cultures. *Soil Sci.* 5: 123-150.

# COLLOIDAL CLAY CULTURES—PREPARATION OF THE CLAY AND PROCEDURES IN ITS USE AS A PLANT GROWTH MEDIUM<sup>1</sup>

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The use of colloidal clay as a substrate for growing plants suggested itself as a transition step from the aqueous to the soil culture methods. Since the speed of chemical activity increases as the total surface of a constant mass increases (or as the mass is more finely divided), it is logical to attempt the analysis of the chemical interrelations of the soil and the plant roots by studying them first by using only the clay, or the most active separate of the soil. It was such reasoning, coupled with the increasing knowledge of the chemical behavior of the colloidal clay, that prompted attempts at growing plants on the colloidal clay fraction of the soil.

In this fraction occur the most rapid chemical reactions natural in the soil, even though these may require several days to reach equilibrium. Nevertheless, such rates are not beyond laboratory patience and may possibly correspond to the reaction rates of the penetrating plant roots, the growing tips of which may be in contact with the clay long enough to exhaust the exchangeable nutrient supply at this slow reaction rate. The isolation of the colloidal clay from the bulk of the soil and its use as a medium for plant culture seemed a logical method for bringing the chemical and biochemical activities of the soil and the plant together. It suggested possibilities for study of the interrelations of these activities with a degree of accuracy and with controls not possible when the entire soil is used. When the entire soil is used, much of its bulk is inactive and reduces the analytical precision below the possibility of encompassing the desired phenomena. It suggested that when once the interactions of root and clay are worked out, and when those of clay and the larger reserve mineral particles are likewise catalogued, then the mechanisms of plant nutrition will be better understood in terms of the soil and its most effective use in crop production.

<sup>1</sup> Contribution from the department of soils, Missouri Agricultural Experiment Station, Journal Series No. 961.

The final procedure for the preparation of the acid colloidal clay on a comparatively large scale and as a nearly continuous process is the result of several years' experiences. From the beginning, when a small three-chamber cell was used, various improvements for greater mass production, speed, and convenience were introduced by the different persons helping with the preparation and concerned with use of the clay. Acknowledgment is gratefully made to R. Bradfield, H. Jenny, C. E. Marshall, E. W. Cowan, E. R. Graham, and E. P. Whiteside as colleagues, and to Ben Moore, Thos. Yager, and other students, both graduate and undergraduate, whose ingenuity in modifying the process was stimulated by the laboriousness of the earlier procedures. The final process is the result of the ingenious efforts of C. M. Woodruff, by whom the present procedure was developed, the apparatus constructed, and the details sketched.

## SEPARATION OF COLLOIDAL CLAY

*Source in soil*

The B-horizon of Putnam silt loam has served as the source of the colloidal material. This stratum contains about 50 per cent clay (<0.002 mm. in diameter) of which about 75 per cent consists of particles <0.2  $\mu$  in diameter. When the clay, as taken from the profile, is kept immersed in distilled water, 100 gm. will contain about 40 gm. of water and 60 gm. of clay, of which about 30 gm. represents the particles of desired size (<0.2  $\mu$  diameter). When 100 gm. of this natural clayey subsoil is added to 3 liters of distilled water for mechanical dispersion, clay of approximately the desired size will occur as a 1 per cent suspension of convenient concentration for electrodialysis.

*Mechanical dispersion of the clay*

Clay and water of the above proportions are put into an earthenware churn, 0.6 gm. of sodium hydroxide is added, and the suspension churned for an hour or more. This is then left to stand so that the larger particles will settle out. Since silt particles (>0.002 mm.) settle through 10 cm. of depth in less than 7 hours at room temperature, it is possible to siphon off this depth of suspension from the top at intervals of 7 hours and obtain particles of only clay size (<0.002 mm.). By allowing longer intervals for settling, still smaller sized particles can be obtained. Thus by controlled time of settling or by use of the centrifuge to remove the fraction of larger clay size, it is possible to obtain a clay suspension consisting of only the finest particles of the soil and therefore those most active in exchange behavior.

*Separation of colloid by means of the centrifuge*

To obtain particles 0.2  $\mu$  and less in diameter, only the well-settled clay suspension is put through a Sharples supercentrifuge. When the suspension is put through at the rate of 10 ml. per second, with a 277-ml. bowl running at 33,000 r.p.m., the particles smaller than 0.2  $\mu$  pass through. The bowl equipped with a celluloid inner sleeve collects the particles of larger dimension. This loaded sleeve must be removed periodically to discard the accumulated clay particles >0.2  $\mu$ . The time interval will vary with the degree to which the coarser clay has been settled out, though removal is usually required after approximately 10 liters of suspension have passed through. The resulting suspension coming over from centrifuging is then ready for electrodialysis.

## ELECTRODIALYSIS OF CLAY

*Cathode and its cell*

As apparatus for the electrodialysis of the colloidal clay, an ordinary 4-gallon stone jar with a drain hole in the side near the bottom provides a sturdy and convenient container. A piece of galvanized hardware cloth, or of fine chicken wire, with 2 to 4 meshes to the inch and fitted snugly inside the jar serves as the cathode. This screen is preferable to a solid metal sheet as a means of preventing

basic ions from collecting between the sheet and the stone jar, for it allows these ions to be carried out by the osmotic flow of water. The assembly of the electrodialysis chamber is obvious from figure 1.

A very serviceable cathode membrane can be had by using No. 4 Bristol board made of wood pulp. It is attached by means of tacks to a circular wood base. Two or more upright strips of wood set firmly into the circular base will serve to support the membrane when wet. One of them can serve as a base to which the ends of the Bristol board may be tacked and where they may be sealed together with molten paraffin. This Bristol board is similarly sealed to the inside top of

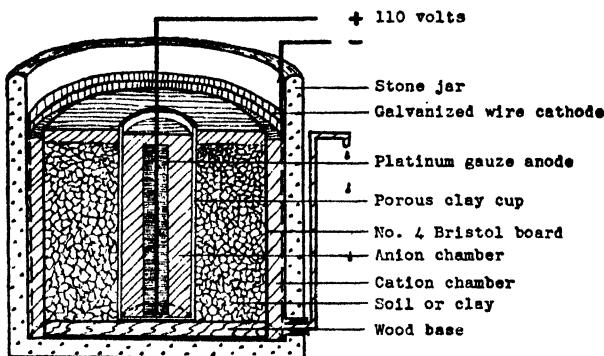


FIG. 1. DIAGRAMMATIC CROSS SECTION THROUGH THE COMPARTMENTS IN THE APPARATUS USED FOR ELECTRODIALYSIS OF COLLOIDAL CLAY

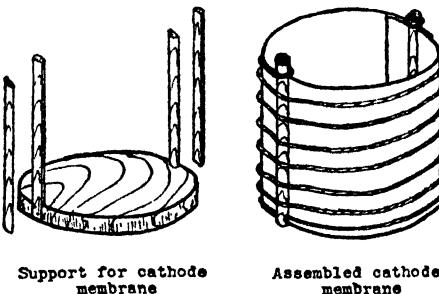


FIG. 2. DETAILS OF WOOD BASE WITH SUPPORTS AND BRISTOL BOARD AS ASSEMBLED TO PROVIDE THE CATHODE MEMBRANE

the circular wood base. Additional support for the membrane may be supplied by wrapping the assembled unit with six to eight turns of cotton twine. The final diameter of the unit should be approximately  $1\frac{1}{2}$  inches less than the interior diameter of the stone jar so as to keep the unit from contact with the wire mesh cathode. The cheap membrane may be discarded and renewed for each batch of clay dialyzed. During dialysis the soluble iron, aluminum, and silica liberated are precipitated as insolubles on the alkaline cathode membrane and thus retard the process. Renewal serves to remove this trouble. The assembly of the simple cathode membrane is shown in figure 2.

*Anode and its cell*

For the anode membrane, a porous porcelain cup with a diameter of  $3\frac{1}{4}$  inches and a height of 8 inches is used. It may be anchored to the wood base by any convenient means. Into this cup is inserted the platinum gauze electrode with 20–25 square inches of total area. The electrode should be attached to a piece of platinum wire heavy enough so that it will not heat when carrying as much as 2 amperes of current. As a control of current flow, a 200-watt light bulb is inserted in series near the anode—or the cathode.

The size of anode cell or chamber recommended and its insertion into the center of the stone jar by locating it on the center of the circular wood base supporting the cathode membrane do not exaggerate the distance between the electrodes but yet give significant volume around the anode chamber to contain the inflowing clay suspension from which the clay will be deposited around the anode membrane. The porous cup allows introduction into it of the distilled water that migrates from the inside of this anode chamber through the porous wall, through the colloidal clay, and overflows with water from the clay suspension into the cathode chamber to siphon off as waste. This is initially alkaline in reaction because of the cations. Complete removal of these from the colloidal clay is indicated by the reaction change to neutrality.

*Continuous operation of the cell*

The suspension of the clay from the centrifuge—or from the suspension of particle size controlled by settling time—is usually taken at a concentration of 1 to 2 per cent. Electrodialysis serves to concentrate the clay, which collects around the anode chamber at the same time that its adsorbed and exchangeable ions are taken out as solution through the respective electrode chambers. When a continuous or an intermittent flow of the dilute clay suspension into the dialysis cell and the necessary flow of distilled water into the anode cup are provided, the clay migrates to the anode membrane, is flocculated, and the excess of clear water flows over the cathode membrane and out of a siphon leading through the hole in the jar wall. The siphon is set at a height to facilitate its automatic functioning in relation to the height of the liquid maintained in the cathode chamber. This siphoning solution carries out the cations.

The anions move into the anion chamber while water is leaving the chamber to migrate in the opposite direction with the cations. Thus, the anions become concentrated. They are allowed to accumulate until the clay cell is half filled before the anode solution is replaced. Its replacement by distilled water reduces the osmotic pressure in the anode chamber and also the flow of electric current to lower the rate of clay movement to the anode membrane. The anion solution should not be replaced oftener than once in 5 to 15 hours so as to prevent lowered rate of cation removal. If the light of the bulb is reduced significantly by changing the solution in the anion chamber, when the current flow had not been reduced before through cation removal—or when the siphoned overflow from the cation chamber was not yet neutral—a drop of sulfuric acid added to the anode chamber will restore the speed of dialysis. The general arrangement of the apparatus for continuous performance by the addition of clay suspension to the dialysis cham-

ber, by the automatic addition of distilled water to the anode chamber, and by the automatic overflow of water and cations from the cathode chamber is shown in figure 3.

#### *Rate of colloidal clay production*

The rate of production of the colloidal clay is dependent on the rate at which it flocculates in the dialysis cell. The rate at which the dilute, centrifuged suspension flows in must be controlled to maintain an overflow of clear water from the top of the cell into the cathode chamber. An adjustable screw clamp on some rubber tubing in the flow line is a possible, though not the most effective, control. A tendency of the clay to stick inside the tubing necessitates occasional massag-

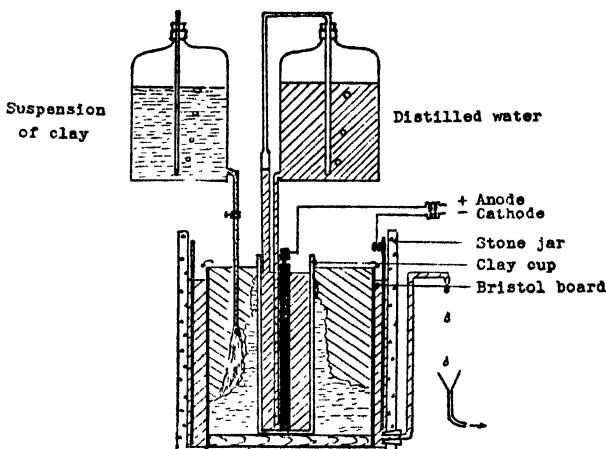


FIG. 3. GENERAL ARRANGEMENT OF APPARATUS AS SET UP FOR CONTINUOUS ELECTRODIALYSIS OF COLLOIDAL CLAY

ing of the tube. The apparatus described has produced 400 to 500 gm. of colloidal clay a week.

To clean the cell of its clay, the clear water above it is siphoned off. The solutions in the anode and cathode chambers are siphoned out at equal rates or in equal unit quantities to prevent irregularities in pressures in the chambers. The hydrated clay as it is adhering to the top of the anode cell and filling the bottom of the dialysis cell can be removed as a pasty mass. It usually contains about 12 per cent of dry matter. Diluting it with an equal volume of water and dispersing it with a motor-driven stirrer result in a homogeneous acid clay suspension of 6 to 7 per cent dry matter. This then can be standardized to a known clay content and to a known exchange capacity per unit clay or unit volume that will serve as the medium into which can be introduced the various ions in adsorbed or exchangeable form for plant growth.

#### STANDARDIZATION OF COLLOIDAL CLAY SUSPENSION

The amount of dry clay per unit volume of suspension is determined by evaporating a measured and weighed volume and determining the dried colloid resulting.

If a particular percentage concentration of colloid is desired the suspension can be diluted or standardized as is commonly done for any standard chemical solution.

Because this acid clay reacts slowly on titration with an alkali, the titration procedure is carried out as a series of increasing volumes of the alkali added to a series of constant volumes of suspension or amounts of clay. For example, 10 aliquots each of 20 ml. of a 5 per cent clay suspension are measured out. If the maximum amount of a standardized saturated solution of calcium hydroxide estimated as necessary to neutralize this amount of clay is 24 ml., for example, then to this series of clays as set up are added a series of 0, 4, 8, 12, etc., to 36 ml. of calcium hydroxide and reciprocally a series of 36, 32, 28, etc., to 0 ml. of distilled water in order to have the final volumes constant and give no change in the clay-water ratio. The maximum amount of hydroxide added will go well past the neutral point. These mixtures are shaken, allowed to stand for some time, and then tested for their pH values. After another time interval they are tested

TABLE 1  
*Composition of electrodialyzed Putnam colloidal clay*

ELEMENT	PER CENT
Silicon.....	23.00
Aluminum.....	12.77
Iron.....	5.73
Potassium.....	0.924
Magnesium.....	0.661
Sodium.....	0.422
Calcium.....	0.177
Manganese.....	0.0188
Carbon.....	1.56
Nitrogen.....	0.157

again. If constant, then by plotting on the abscissa the milligram equivalents of hydroxide added, and the resulting pH values on the ordinate, one can determine the milligram equivalents required to bring a unit mass of the colloidal clay to a pH of 7.0, or can thus determine its exchange capacity as milligram equivalents per gram or per hundred grams of the dry clay.

#### PREPARATION OF PLANT GROWTH MEDIUM FROM COLLOIDAL CLAY

##### *Stability of colloidal clay*

The use of the colloidal clay as a medium for plant growth is founded on the assumption, of course, that the clay will deliver only the nutrients carried by it in their exchangeable forms. The clay itself is relatively stable, as the process of electrodialysis suggests. In a long period of contact with plant roots, however, it may break down to give up enough iron to supply the plants with this element, or of some other elements not detected by common methods of chemical analysis and used in extremely small amounts by plants. An analysis of the clay, including its carbon and nitrogen contents, is given in table 1. Since the amounts of

these two organic elements have been found to be nearly the same in the colloid before growth of a crop as they were after recovery and electrodialysis following growth of three successive crops, it is believed that the carbon and nitrogen are relatively stable in the colloid and are contributing very little to the growth of the crop. The colloid is considered, therefore, a relatively indifferent medium so far as its contribution of nutrients other than those exchangeable is concerned.

*Calculation of amounts of clay and nutrients needed*

In making up the medium for plant growth, one must first estimate the amount of each of the nutrients required for the amount of plant growth anticipated. This estimate must take into account the fact that the plant does not exhaust to the same degree each of the exchangeable elements adsorbed on the clay colloid, nor does it exhaust them completely. The amount of clay needed to hold these total amounts of nutrients in exchangeable form and yet not be completely neutralized by their adsorption must then be determined. This amount of clay is then measured from the standardized solution and the respective cations are

TABLE 2

*Milligram equivalents of hydrogen and corresponding amounts of nutrients to be titrated on colloidal clay for a series of pot cultures for soybean growth*

POT NUMBER	CALCIUM m.e. mgm.	MAGNESIUM m.e. mgm.	POTASSIUM m.e. mgm.	PHOSPHORUS m.e. mgm.	BARIUM m.e. mgm.
1	10 200.4	5 60.8	0 0	3.68 38.0	15 1030.2
2	10 200.4	5 60.8	5 195.5	3.68 38.0	10 686.8
3	10 200.4	5 60.8	10 391.0	3.68 38.0	5 343.4
4	10 200.4	5 60.8	15 586.5	3.68 38.0	0 0

titrated thereon to be carried by the clay colloid as it is mixed into quartz sand to serve as the growth medium for the plants.

As an illustration of a series of pot cultures set up for growing soybeans, the milligram equivalents as hydrogen and the corresponding milligrams of the different elements are given in table 2. Since only the cations represent adsorption on the negative colloid, the total exchange capacity needed is that sum for calcium, magnesium, and potassium at the maximum in this series, which was varying the potassium and using barium as its reciprocal. The total exchange capacity here amounts to 30 m.e. With a clay of which the exchange capacity is 65 m.e. per 100 gm., then  $30/65 \times 100$  gm. will require 46.15 gm. of clay. If a standard clay suspension with 5 per cent colloid is used, this amount of colloid will be contained in 923 ml.

*Titration of nutrients on the clay*

In preparing the clay medium for culture 1 in table 2, for example, the first element added is barium as barium hydroxide. Since this is not a nutrient, it is added first or when the clay is still most acid. Next, a standard solution of monocalcium phosphate is added to supply the desired amount of phosphorus. Magnesium oxide is then added to provide the necessary amount of magnesium.

Calcium hydroxide is next added to supplement the calcium added as phosphate and to bring the total calcium to the amount needed. The potassium is finally added as the hydroxide, or possibly as the sulfate to supply this latter nutrient and not push the reaction of the clay too near to neutrality.

Between the addition of one cation and that of the next, a time interval is allowed, and the suspension is stirred mechanically. The pH is then measured to learn whether the change due to cation addition corresponds to the calculated theoretical change. If not, additional time will be necessary, so that all of the added cation will be adsorbed before the next one is added. In this manner each culture is built up with each nutrient completely adsorbed individually. This condition can be tested finally by filtering the colloidal clay suspension through a collodion membrane under pressure and testing the clear filtrate for the presence of cations. The final culture should still be slightly acid, and no cations should occur in the filtrate. Since the final volume of clay suspension may be more than can be taken by the amount of quartz sand desired, it can be evaporated at room

TABLE 3

*Consumption of supplies of exchangeable nutrients by a soybean crop from colloidal clay culture*

(Resulting from nutrients supplied in table 2.)

POT NUMBER	EXCHANGEABLE NUTRIENTS CONSUMED				
	Calcium per cent	Magnesium per cent	Potassium per cent	Phosphorus per cent	Barium per cent
1	27.6	78.5	49.6	45.2	11.8
2	28.9	71.0	59.8	44.7	16.7
3	31.8	61.9	65.0	50.5	24.3
4	33.8	52.7	61.6	50.5	....

temperature and under forced air circulation until the volume can be uniformly and completely contained within the sand volume. When the culture is regulated to contain the desired amount of moisture, it is ready to be planted.

#### COLLOIDAL CLAY CULTURES PERMIT ACCURATE CONTROLS OF FERTILITY

The colloidal clay cultures are particularly serviceable in plant growth studies in that they permit of accurate chemical scrutiny of seed and soil at the outset and of crop and soil at the end of crop growth. By this means one is able to study the movements of plant nutrients from the clay into the plants and from the plants into the clay, as may be the case under nutrient deficiencies. Degrees of exhaustion of the different nutrients from the clay by particular crop growth, the influences of the nutrients on one another at different levels, and many other interesting relations of soil fertility to the plants, and *vice versa*, can eventually be understood through wider use of the technique of growing plants in colloidal clay cultures. These can be either the simple aqueous suspensions of the clay, or mixtures of clay and quartz sand or of clay and nutrient-bearing minerals. As

a simple illustration of the degree to which the exchangeable nutrients may be taken from the clay by plants, the exhaustion of nutrients by a soybean crop is shown in table 3.

The possibility of getting a chemically accurate balance sheet of nutrient supplies both before and after the crop growth is one particular value of this technique which alone warrants its wider use in research in soil fertility, in plant physiology, or in any study concerned with the interrelationships of the soils and the crops they produce. As a refined step in soil research it can do much to bring about our better understanding of many agronomic matters.



## POT METHOD FOR SOIL CULTURES

W. H. MACINTIRE AND S. H. WINTERBERG<sup>1</sup>

*The University of Tennessee Agricultural Experiment Station*

The development of new processes and the improvement of old methods for the conversion of mineral phosphates to effective phosphatic fertilizers constitute an objective of The Tennessee Valley Authority. The Agricultural Experiment Station of The University of Tennessee has collaborated in the furtherance of that objective through chemical, biochemical, and x-ray studies of the properties and behavior of products and by-products from the laboratories and pilot plants of the Authority. To establish the fertilizer effectiveness of an experimental material and warrant its production in quantities requisite for field trials, it was expedient to integrate the analytical indications with the response by plants in pot cultures. The presently described greenhouse facilities, therefore, were provided by the Authority to obtain pilot information relative to the responsiveness of the experimental products on soils of major occurrence. Particular emphasis was placed on the several factors of particle size, rate and mode of incorporation, rôle of concomitant components, fixation, and compatibility with other fertilizers and with variant liming materials. It was not assumed that the response obtained under controlled conditions in the greenhouse would give unqualified confirmation of the indications obtained by the chemical analyses and Neubauer tests. It was recognized that ultimate answers as to practical utility would come from field trials and test demonstrations. It was believed, however, that the results of the pot tests would serve to establish certain principles and register probabilities in advance of results obtainable through trials on a more extensive scale.

### EQUIPMENT

#### *Buildings*

The structure is 30 by 60 feet and has floors on two terraces, as shown in figure 1. It is fronted by a wire-cloth-enclosed solarium, the floor of which extends into the floor of the greenhouse. The noncorrosive metal skeleton of the superstructure rests upon heavily reinforced 10-inch concrete walls that stand 2½ feet above the greenhouse floor. The spaces between the horizontal steel-concrete girders of the upper floor are insulated by fill-ins of clay tile.

The basement affords facilities for the sieving, mixing, and potting of soils, for operative equipment and storage, and for shower, lavatory, and bedroom. Ac-

<sup>1</sup> Acknowledgment is due T.V.A. personnel for drafting details and specifications for the essential equipment and for erecting the concrete structure. In particular, appreciation is expressed to C. E. Norton for supervision during construction and for devising and making the cam-operated trucks. The custom-built superstructure and solar shades were purchased from The William H. Lutton Company, of Jersey City, New Jersey. Collaboration in the inauguration of the experimental work was accorded S. F. Thornton, then of Purdue University.

cess is by exterior concrete steps at both ends of the structure. The outdoor end-platforms facilitate delivery of soil and also afford a repository for stoker clinkers.

Heating is by the circulation of warmed and humidified air, in lieu of steam-piping. The boiler is stokered from a 20-ton bin, to which coal is chuted through an orifice in the floor of the solarium. The conditioned air is propelled by a large fan driven by a  $\frac{1}{4}$ -horsepower motor, and is introduced downward through two insulated rectangular goose-neck metal ducts. These terminate at points 10 feet above the floor and 16 feet from its ends and have dispersive plates at their terminals, as shown in figure 3. The air is returned to the conditioning equipment in the basement through four floor grates and thence via rectangular metal ducts suspended from the ceiling of the basement. The resultant circulation assures greater uniformity of temperature and humidity at the table level throughout the greenhouse than when heating is by means of pipes along the walls.

Ventilation is by means of two full-length sash that hinge outward from the apex of the roof. Each of these is operated by a screw gear propelled by a single wheel 4 feet from the floor level. Ventilation is afforded also by means of adjustable sash, which constitute the upper half of the perpendicular glass housing that extends from the top of the concrete walls to the bottom of the curvature of the stationary glass roof, front and rear. The two frontal segments of the wall sash are operated by separate gear-wheels, whereas the full-length rear sash is geared to a single wheel.

Shading of the superstructure is provided by means of "solar shades." These can be rolled from the apex of the roof down to the top of the front and rear walls, and *vice versa*, and are removed and stored over winter. During summer, the glass-panel ends of the superstructure are coated with an easily removable coating of light green waterpaint.

Rainwater from the roof is caught by an exterior gutter, which extends along the lower terminus of the glass curvature, and is deflected from the face of the concrete walls and from the floor of the solarium. The gutter is joined to copper tubes that lead to an interior trough, which delivers to a pair of 4-inch drainpipes in the basement and into the sewer. This provision protects the exterior of the concrete walls from discoloration. Condensations from the ceiling and interior faces of the sash likewise are caught in a small metal trough that extends along the base of the glazing and to the drainpipes.

The solarium is 18 by 90 feet. It is framed by 1½-inch galvanized iron piping and is screened by  $\frac{1}{2}$ -inch-mesh wire "cloth" (fig. 2). Its floor slopes from the walls of the greenhouse to frontal gutters and is unobstructed by uprights. In case of snow or sleet, however, flanged-end pipe uprights can be placed quickly to support the horizontal piping and wire cloth of the roof.

#### *Tables and transfer trucks*

Tables and transfer trucks are used instead of either cars on stationary tracks or individually castored trucks having handles. The table frames and legs are of oak and are 2 feet in height. The tops are of cypress and are  $2\frac{1}{2}$  by 5 feet. It is believed that tops made from 5- or 6-inch boards with  $\frac{1}{2}$ -inch spacing would be

preferable to the solid platform. Fifty-nine tables of 12-pot capacity accommodate 708 cultures.



FIG. 1. VIEW OF WEST END OF GREENHOUSE AND SOLARIUM  
Lysimeter installation in the foreground



FIG. 2. VIEW SHOWING SOUTHWEST CORNER OF SOLARIUM, SOLAR SHADES, AND FRONTAL ENTRANCE TO GREENHOUSE

The transfer truck has rubber castors and an elevated cross-bar handle at one end. It is provided with hand-and-foot operated cams, by means of which a table is lifted or lowered for transfer. Using a pair of trucks, two workers can transfer the 59 tables to or from the greenhouse in 30 minutes. The elimination



FIG. 3. INTERIOR VIEW SHOWING ONE GOOSE-NECK AIR DUCT, TABLES OF CULTURES, TABLE SHIFTER, SCALES, AND TWO TRUCKS

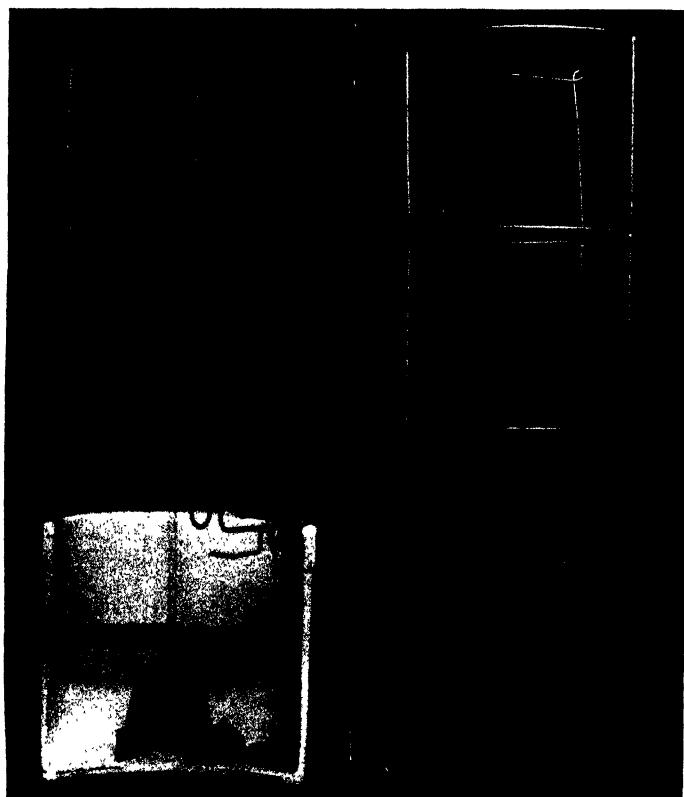


FIG. 4. CROSS SECTION OF 2-GALLON POT AND INVERTED CLAY POT GLASS TUBE, ASPHALTUM-COATED WIRE-CLOTH DISC, AND WIRE CAGE

of rubber castors on individual tables effects substantial savings in initial outlay and in upkeep. Alignment and close spacing of the tables in greenhouse and solarium is facilitated by use of a small auxiliary truck, devoid of handles and operated by a detachable lateral lever (fig. 3). A special truck-table is used to afford an adjustable-level platform for the metric scale, on which the cultures are weighed.

#### *Pot cultures*

The crops are grown in 2-gallon undrained glazed earthenware pots, and moisture requirements are supplied by the use of distilled water exclusively. For rock-derived soils, an air-dry constant of 8.2 kgm. per pot is used. As shown in figure 4, access of air is provided by means of a glass tube that extends vertically from near the bottom of the pot and through the hole of an inverted 3-inch clay pot to a height 3 inches above the surface of the soil. This tube can be adjusted vertically by means of a rubber collar that catches on the edge of the hole in the bottom of the inverted pot. It is used also in the watering of certain cultures, when their surfaces are to be restricted in moisture content.

The upper and lower zones of soil are separated by means of a disc of  $\frac{1}{4}$ -inch asphaltum-coated galvanized wire-cloth. Plant support is provided by means of a 10-gauge spot-welded galvanized wire cage that is clipped over the rim of the pot. The cage appropriate for either red clover or lespedeza can be heightened by the attachment of a second section to accommodate such crops as Sudan grass.

An identical over-all initial weight of cultures containing a constant weight of soil would facilitate and expedite the subsequent weighings to determine the amounts of water to be restored to the cultures. Unfortunately, however, this ideal setup is not attained, since pots of uniformity in weight are not obtainable. This difficulty is met by segregating the pots into groups, according to weight, and then bringing the pots of a particular group to a common weight by adding inert gravel. The pots of a group are given an identical prefix to their numerals that designate experimental treatments. This prefix indicates the common weight to which the cultures of the group should be watered.

#### OPERATIVE TECHNIQUE

##### *Soil selection and preparation*

Choice of soils is based upon extent, type, reaction, and responsiveness to phosphates. Identification by survey personnel is sought before a soil is included in an extensive setup. Only the plowed zone, or A horizon, is used; forest soils and those adjacent to gravel are avoided. The soil from an identified locale is trucked to the greenhouse and there sifted through a  $\frac{1}{4}$ -inch wire screen and mixed to uniformity. Its pH is determined and its responsiveness to a specific fertilizer element is established by either Neubauer tests or pilot pot trials, or by both procedures.

##### *Fertilizing and liming*

In most cases, fertilizers are incorporated on the basis of pot area in relation to an acre, rather than to 2,000,000 pounds of soil. Every treatment is made in

duplicate, triplicate, or quadruplicate. The moist soil and fertilizers required for multiples of a given treatment are mixed to uniformity in the device described by Cook (1) and then are divided equally among the several pots. When no incorporation is to be made with the lower zone, that zone is placed and topped with a wire-cloth disc for zonal demarcation.

The experiments have dealt chiefly with experimental phosphatic concentrates—triple superphosphate, calcium and potassium metaphosphates, dicalcium phosphate, fused tricalcium phosphate, and magnesia-fortified materials (8, 10). In most cases, these have been incorporated with the upper 3 inches of soil, to simulate an inworked broadcast. In some comparisons, however, the phosphates have been placed in layers or incorporated with the full depth of soil. Usually the incorporations have been at the rate of 40 pounds of total  $P_2O_5$  per acre, although rates of 80, 160, or 320 pounds also have been used. In the correlation of plant response with chemically measured "availability," the incorporations have been based upon percentage content of available  $P_2O_5$ .

Objectives dictate whether incorporations of phosphates of variant chemical type and physical properties shall be made with full depth of soil or in the upper or the lower zone or in layers. The objectives also dictate whether the incorporations of phosphates shall precede, accompany, or follow the incorporation of the liming materials, as well as the period the prelimed soil shall be "aged" (2, 3, 4). When successive crops are to be grown, fertilizer and soil amendment repetitions are governed by the extent of plant response, by analytical determination of uptake of nutrients, by changes in soil reaction, and by experimental objectives. In particular instances, successive incorporations of phosphates include a replacement of the amount of  $P_2O_5$  removed by previous crops.

When experiments involve comparisons of experimental phosphates, the requirements for other nutrients are provided through additions of potassium sulfate, magnesium in appropriate form, and by a composite solution of zinc, manganese, copper, and boron compounds. When comparisons of carriers of nitrogen are not involved, that element is supplied by ammonium nitrate. Although a constant is used in the initial applications, subsequent additions are variant and are governed by the quantities necessary to maintain a good green color in the plants of the respective cultures. Particular care is taken to assure that the nitrate additions to a succession of crops will not be so heavy as to cause a detrimental accumulation of salts.

Liming is almost invariably beneficial in the growing of legumes on soils of this area and is essential in all cases after two to three crops are grown on unlimed potted soils. The technique for the incorporation of liming materials is governed by the specific objective. In some experiments the incorporations are allowed to react with the soil before the phosphates and other fertilizer materials are incorporated. In such cases, each bulk of limestone, dolomited, or slagged soil is spread to a depth of 4 inches and wetted once a week to about 50 per cent of water-holding capacity during 15 to 30 days. The limed soil then is allowed to dry to a condition suitable for a repetition of the sieving and mixing. In other cases, the liming materials and fertilizers are incorporated simultaneously, with

the full depth of soil or with the upper half. When more than four successive crops are to be grown, it is believed that the liming materials should be incorporated full depth. In experiments that involve variance in particle size, rate, and zone of incorporation (2, 5, 6, 8, 9), the quantities of the liming materials to be incorporated initially and subsequently are governed by exchange capacity, by the initial and the altered pH, and by observations as to amounts that induce repressive effects. Unless the particular objective dictates otherwise, the limestone and dolomite used are 100-mesh materials and all liming incorporations are based on CaCO<sub>3</sub>-equivalence.

#### *Growing and harvesting*

Choice of the initial crop, and of successions, is governed by season, length of day, adaptation, resistance to disease, and suitability in general. At times alfalfa, sweet clover, lespedeza, and soybeans have been utilized, but most of the comparisons have been made by the use of either Sudan grass, red clover, or rye grass. The seeding technique varies and will be detailed for the several crops.

In the summer, the cultures are trucked from the greenhouse to the solarium daily, with variation in the placement of the tables. During winter they are shifted periodically indoors, to obviate the effect of any unavoidable variance in shading, and every pot is rotated 180° once a week. In some tests, as many as ten successive crops are grown. In such cases, the soil above the wire-cloth disc is removed after each harvest and then is sieved, mixed, and returned.

Sudan grass has been found excellent for greenhouse cultures in spring, summer, and fall. It is responsive to phosphate and tolerant to liming. In the preparation of the seedbed, approximately 325 gm., or 200 cc., of the air-dry soil is removed and the remaining soil is brushed level and tamped lightly by means of a wooden disc. About 100 seeds then are distributed and the removed portion of soil is returned, leveled, and covered temporarily with a 25-cm. disc of filter paper, which protects the seedbed against disruption by the initial watering.

Moisture content of about 35 per cent of water-holding capacity has been found optimum for the germination of Sudan grass. The seedlings are thinned to 25 plants per pot after a week's growth, and to 20 plants after 2 weeks. The cultures are weighed weekly, or more often, during the growing period, and the moisture content is brought to 50 per cent of the water-holding capacity. When the plants are large and the atmosphere is clear and hot, watering every other day, or even daily, may be necessary. In such cases, the respective needs for the watering of the cultures of small, medium, and large growth are determined by weighing several pots as representative of each order of growth. When heads appear, the cultures are photographed and the plants are harvested, allowed to dry, cut to small pieces, heated 3 hours at 90°C., and weighed.

The plants are protected against aphids by periodic fumigation with "cyanogas," and against thrips through spraying with a solution prepared by dissolving 2 ounces of tartar emetic and 3 of brown sugar in 3 gallons of water.

Red clover is well adapted for studies of phosphates and liming materials during the winter and spring season. The seeds are treated with a copper compound

to prevent postemergence damping-off, and 75 to 100 per pot are used. Protection against anthracnose and mildew has been provided by the use of seed derived from open-pollinated disease-resistant breeding plants grown on plots of the department of plant pathology.

The seedbed for clover is prepared and watered initially as prescribed for Sudan grass. To keep the surface of the soil relatively dry during the early growth of the seedlings and thus minimize damping off, the cultures are watered through the glass tube (fig. 4) during the 4- to 6-week period following emergence. The supplying of water through surface applications then is admissible. After 15 to 30 days' growth, the plants are thinned to 12 per pot. When maturity of stems is attained by the plants that show the best growth, the clover crop is harvested and handled in the manner prescribed for Sudan grass.

Aphids and white flies have been controlled by fumigation, and protection against the red spider has been afforded by the use of the prescribed tartar emetic solution.

Rye grass is an excellent plant for fall and winter cultures and grows well on acidic soils. The technique prescribed for seeding, growing, harvesting, and insect control for Sudan grass is applicable also for rye grass. The seedlings are thinned to 25 plants per pot after about 3 weeks.

Robber cropping. When the major objective is a comparison of nitrogen carriers (7), it is deemed essential that the nonlegume test crop be preceded by a different nonlegume, so that the soil will be depleted of nitrates that may have accumulated during the preparatory operations and as a consequence of advanced incorporation of P and K and liming materials. When its growth indicates depletion of the soil-derived nitrates, the robber crop is removed and the soil is conditioned for the successive crop that is to measure the effectiveness of the applied or incorporated nitrogenous compounds.

#### *Provision for corollary chemical determinations*

In certain experiments, crop composition is determined. When an objective calls for the determination of the transitions that carbonates and silicates undergo after incorporation, supplemental units of the potted soils are provided to ensure samples for carbonate determinations and Neubauer tests. In such cases, the fallow soil of the supplemental pots is quartered by uprights and the several segments are utilized as periodic samples. In selected instances, the pot cultures are supplemented with identicals in outdoor lysimeters, which afford rainwater leachates that register the migration of Ca, Mg, K, PO<sub>4</sub>, fluorides, sulfates, and nitrates. The greenhouse equipment has been implemented in the carrying out of some of the studies reported in certain of the citations.

#### REFERENCES

- (1) COOK, R. L. 1939 A mixer and sampler for greenhouse soils. *Jour. Amer. Soc. Agron.* 31: 171-174.
- (2) MACINTIRE, W. H., ET AL. 1940 Nature and liming value of quenched calcium silicate slag. *Soil Sci.* 50: 219-237.

- (3) MACINTIRE, W. H., AND HATCHER, B. W. 1942 The beneficial effects of preliming upon  $\text{PO}_4$ -uptake from incorporations of water-soluble calcium phosphate. *Jour. Amer. Soc. Agron.* 34: 1010-1015.
- (4) MACINTIRE, W. H., AND HATCHER, B. W. 1942 Evidence in support of a new concept as to the end-product of superphosphate in limed soils. *Soil Sci.* 53: 43-54.
- (5) MACINTIRE, W. H., ET AL. 1942 The fluorine content of certain plants fertilized with phosphates and slags carrying fluorides. *Indus. and Engin. Chem.* 34: 1469-1479.
- (6) MACINTIRE, W. H., AND WINTERBERG, S. H. 1943 Quenched calcium silicate slag—a by-product substitute for limestone and superphosphate. *Tenn. Agr. Exp. Sta. Bul.* 184.
- (7) MACINTIRE, W. H., ET AL. 1943 Response of sudan grass to ammonium hydroxide in pot cultures. *Soil Sci. Soc. Amer. Proc.* 8: 205-210.
- (8) MACINTIRE, W. H., ET AL. 1944 Fused tricalcium phosphate: relation of degree of defluorination to fertilizer value of quenched fusions of rock phosphate. *Soil Sci.* 57: 425-442.
- (9) MACINTIRE, W. H., ET AL. 1944 Industrial precipitated tricalcium phosphates—variance in chemical, structural, and fertilizer properties. *Indus. and Engin. Chem.* 36: 547-552.
- (10) WALTHALL, J. H., AND BRIDGER, G. L. 1943 Fertilizer by fusion of rock phosphate with olivine. *Indus. and Engin. Chem.* 35: 774-777.



# INTENSIFIED CROPPING TO DIAGNOSE MINERAL ELEMENT DEFICIENCIES, A METHOD TO DETERMINE RELATIVE BORON CONTENTS OF SOILS

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Intensive cropping of a soil to which have been added all the essential mineral elements, except the one under investigation, provides a means of classifying soils on the basis of their relative contents of the element omitted from the nutrient solution. The success of such a procedure depends greatly upon the choice of an indicator crop. Through differences in composition or in growth or by foliar symptoms, the plant must provide suitable criteria of variable quantities of the element absorbed from the soil. It is essential also that the indicator crop be sensitive to the element in concentrations which represent a range of practical usage.

A greenhouse procedure designed to determine the relative boron contents of soils is presented herein. The method itself, as well as supplementary experiments dealing with variations in technique, was presented in an earlier paper (2). The sunflower is used as an indicator plant, previous work having shown it to be superior to tobacco, sugar beet, or nasturtium (1). The criterion used to measure the relative boron contents of the soils is the age of the sunflower plant at the time boron-deficiency symptoms first appear; thus weighings and chemical analyses of plant tissue can be omitted.

The procedure is patterned after that of Stephenson and Schuster (4), who used the sunflower plant to determine the needs of boron, sulfur, potassium, phosphorus, and nitrogen in certain orchard soils. In their work, growth differences were used as the criterion of an element deficiency.

## GREENHOUSE PROCEDURE

Soil samples were collected from a roughly rectangular hole approximately 18 by 24 inches and 2 feet deep. From each of the four faces, thin vertical slices of soil from the 0-12- and 12-24-inch depths were obtained and composited to make a sample of  $\frac{1}{2}$  to 1 gallon. After air-drying, the soil was screened through a quarter-inch sieve to remove large stones and roots. One-pound portions were placed in No. 1 tall reenameled charcoal cannery tins obtained from the American Can Co.

To supply standards, a series of quartz sand cultures to which different increments of boron were added as boric acid was included in each experiment. The sand was washed with 30 per cent HCl for 48 hours, then with distilled water until no  $\text{Cl}^-$  was detectable. Twelve 1-pound portions, duplicates for each of six boron levels, were weighed out.

<sup>1</sup> Though the author is now agronomist, The Rockefeller Foundation, Mexico, D. F., the experimental work was conducted at the University of Idaho and Cornell University.

Ten seeds of a Mammoth Russian variety of sunflower purchased locally were planted in each container. The appropriate moisture level was maintained by adjusting the quantity of water to conform to the fixed quantity of nutrient solution given in table 1 and the total weights given in table 2.<sup>2</sup> These weights were increased by 10-gm. intervals two or three times during the growing period to compensate for increased plant weights and greater moisture losses. Water was added in the mornings, and unless unusually high temperatures prevailed, one watering daily was sufficient. An attempt was made to maintain a greenhouse temperature of 65 to 70°F.

TABLE 1  
*Schedule of nutrient solution additions*

TIME FOLLOWING PLANTING	NUTRIENT SOLUTION ADDED	TIME FOLLOWING PLANTING	NUTRIENT SOLUTION ADDED
days	ml.	days	ml.
0	..	13	10
8	10	14	10
9	..	15	..
10	10	16	25
11	10	17	..
12	..	18	25*

\* Twenty-five milliliters every other day for the remainder of the experiment.

TABLE 2

*Amounts of water added to soils of three textural groupings and total weights of filled containers*

TEXTURAL GROUPING OF SOILS	WEIGHT OF AIR-DRY SOIL	WEIGHT OF WATER TO BE ADDED	WEIGHT OF CONTAINER (APPROX.)	TOTAL WEIGHT
Sands (including quartz sand) .....	454	53	73	580
All soils lighter than heavy clay loams	454	73	73	600
All remaining soils heavier than heavy clay loams .....	454	93	73	620

The composition of the nutrient solution is presented in table 3. A stock solution containing 5 gm. of ferric tartrate per liter was used as a source of iron for the quartz sand cultures. At intervals of 2 or 3 days, 1 or 2 ml. of this solution was added with distilled water.

The cultures were thinned to five uniform seedlings at least by the ninth day. Boron was added to the quartz sand series according to the schedule in table 4. As solution A contained 0.6483 gm. H<sub>3</sub>BO<sub>3</sub> per liter, 1 ml. added to 1 pound of sand supplied 0.25 p.p.m. B on a weight basis. Solution B was made by diluting solution A so that 1 ml. added to 1 pound of sand supplied 0.01 p.p.m. B.

<sup>2</sup> The substitution of 25 ml. of complete nutrient solution for the last portion of the 53 gm. of distilled water added to the quartz sand on the day of planting was found on repeated trials to decrease injury from the "damping-off" organism.

TABLE 3  
*Composition of nutrient solution*

SALT	STOCK SOLUTION CONCENTRATION	QUANTITY PER LITER OF NUTRIENT SOLUTION
KH <sub>2</sub> PO <sub>4</sub>	1.0M	5
K <sub>2</sub> HPO <sub>4</sub>	0.2M	5
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1.0M	7
Cu(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	1.0M	7
NaNO <sub>3</sub>	1.0M	7
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.20 gm./l.	
ZnCl <sub>2</sub>	0.10 gm./l.	
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.02 gm./l.	5

TABLE 4  
*Schedule of boric acid additions to quartz sand series*

TIME FOLLOWING PLANTING	H <sub>2</sub> BO <sub>3</sub> SOLUTION	BORIC ACID SOLUTIONS ADDED*					
		0.0 p.p.m. B	0.05 p.p.m. B	0.10 p.p.m. B	0.30 p.p.m. B	0.50 p.p.m. B	1.00 p.p.m. B
days	cm. <sup>-3</sup> /l.	ml.	ml.	ml.	ml.	ml.	ml.
9	(B) 0.02593		5	10	10	10	10
12	(B) 0.02593				20	20	20
17	(B) 0.02593					20	20
29	(A) 0.6483						2

\* p.p.m. B = final concentrations of boric acid, weight basis.

TABLE 5  
*Ages at which initial symptoms of boron deficiency appeared in each of five plants per culture*

Planting date April 20, 1941—sunlight + fluorescent

CULTURE DESCRIPTION (Quartz sand)	AGE OF PLANT				
	No. 1	No. 2	No. 3	No. 4	No. 5
days	days	days	days	days	days
0.0 p.p.m. B	11	11	11	11	12
	11	11	11	11	11
0.05 p.p.m. B	19	20	20	20	21
	22	22	23	23	24
0.10 p.p.m. B	22	24	24	24	25
	24	24	24	25	26
0.30 p.p.m. B	32	32	33	33	33
	33	34	31	36	36
0.50 p.p.m. B	38	38	39	40	41
	38	38	39	39	41
1.0 p.p.m. B	45	45	46	47	48
	44	44	45	45	46

The criterion of the boron status was the age of the cultures the day the first one of the five plants showed the initial symptoms of boron deficiency and is referred to as the "age value" in this report. The term "average age value" is the arithmetical mean of the age values of the 0-12-inch and the 12-24-inch horizons for a given profile. Data in table 5 show that the four remaining plants in a culture produced deficiency symptoms quickly after the first. They were usually not recorded or considered in the interpretation of results. The age value was found to be reproducible in different aliquots of the same medium and capable of detecting the initial 0.05 p.p.m. B by an age value difference of around 10 days

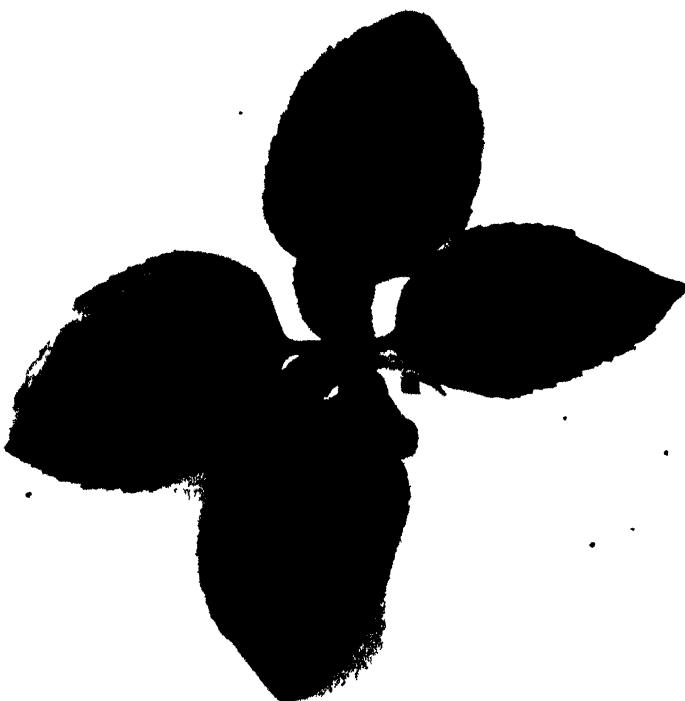


FIG. 1. SUNFLOWER PLANT SHOWING INITIAL SYMPTOMS OF BORON DEFICIENCY

(table 5). This corresponds to an absolute quantity of only 23 γ boron in a pound of sand. In an experiment not reported here, 0.02 p.p.m. B (9 γ per pound) was detected by a difference of 7 to 8 days. The sunflower is less sensitive to small amounts of boron at higher concentrations.

Boron-deficiency symptoms of the sunflower are definite and easily recognizable even in early stages of development. It is on this fact that the value of the plant as a criterion of the boron content of soils as employed in this method is dependent. Since special emphasis is placed on the first indication of the deficiency, a plant showing initial symptoms is illustrated in figure 1, and the following description is presented to supplement those already published (1, 3):

The first symptom is usually a yellowing at the base of the youngest leaves, which may or may not cup downward at the edges the first day. Usually, though not always, the pair of leaves just older than the newest ones show an abnormal shiny green appearance when held in direct light and turned at various angles. They themselves may be somewhat yellowed at the base at the time this shiny appearance is evident toward their centers. Cupping and curling usually appear a day or so later than the first indication of yellowing.

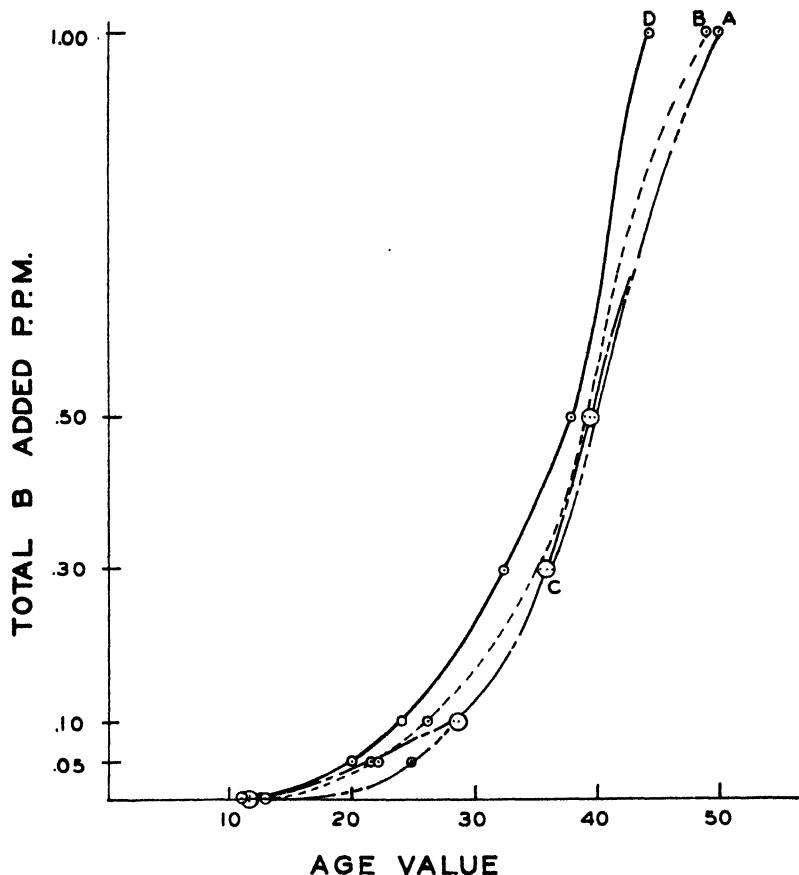


FIG. 2. AGE-VALUE CURVES OF QUARTZ SAND CULTURES TO WHICH DIFFERENT INCREMENTS OF BORON WERE ADDED

- A—Sunlight only, planted March 10, 1941
- B—Sunlight plus fluorescent, planted March 10, 1941
- C—Sunlight only, planted April 20, 1941
- D—Sunlight plus fluorescent, planted April 20, 1941

and shininess. The later stages of a dead terminal and rosetting are well known and, as far as this test is concerned, provide only confirmation of the earlier diagnosis.

The quartz sand cultures receiving no boron developed definite symptoms so rapidly that great confidence could be placed in the exact day of the recorded age value. In those soils which produced boron-deficient plants within 30 to 35 days

the symptoms usually developed rapidly enough after the first indication to be accurate in the recording within 1 or 2 days. A 2-day variation corresponds to a difference of approximately 0.02 p.p.m. B in the 20-day range or of 0.05 p.p.m. B in the 32-day range. Slow-growing plants are not accurate indicators; if the soil does not support rapid growth, it cannot be "analyzed" by this method.

To eliminate so far as possible the effects of certain variations in environment from one growing period to another, age values for soils were interpreted in relation to the quartz sand calibration series. Boron concentration-age value curves for quartz sand are shown in figure 2. At any of the boron levels, age values were found to be reproducible. In these experiments, the use of supplementary light<sup>3</sup> had but little effect. The appearance of the plants near the end of one growing period is shown in figure 3.

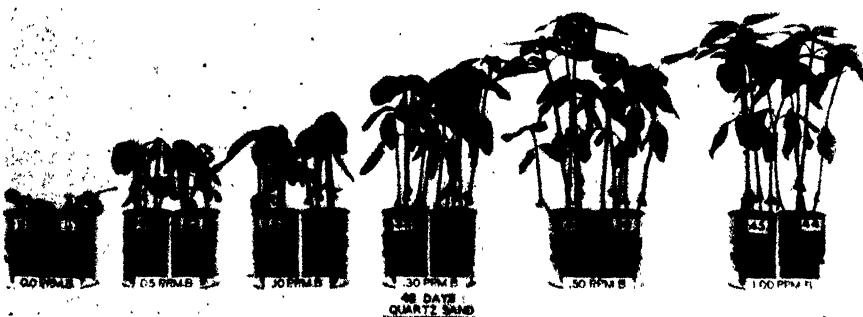


FIG. 3. AGE VALUES AND APPEARANCE OF SUNFLOWERS GROWING IN QUARTZ SAND TO WHICH THE INDICATED QUANTITIES OF BORON HAD BEEN ADDED

#### APPLICATION OF THE METHOD TO SOME SOILS OF IDAHO AND NEW YORK

Supplementary field experiments which were conducted in Idaho and in New York and reported in detail elsewhere (2) showed that marked boron-deficiency in alfalfa was indicated by an average age value below 28. This corresponds approximately to the 0.10 p.p.m. B level in the quartz sand calibration series. Moderate deficiency was found in fields represented by soil samples with an average value between 28 and 36, corresponding approximately to the 0.10 p.p.m. B and 0.30 p.p.m. B concentrations in the quartz sand series respectively. For values above 36 days, or if the 0-12-inch horizon produced healthy sunflowers and the 12-24-inch produced deficient sunflowers, it is doubtful whether a significant return could be expected from borax applications. These limits are relative, and in all probability modifications in age-value interpretation would be necessary under a different set of conditions. A significant feature is the parallelism between the range of boron contents, or the degree of deficiency in the field, and the age values of the corresponding soils as determined by this procedure.

An excellent correlation was obtained between age values of samples from apple orchards in New York and boron-deficiency records<sup>4</sup> on apples. Sunflowers

<sup>3</sup> Fluorescent light to furnish 850 to 900 foot-candles for 13 hours each day.

<sup>4</sup> From A. B. Burrell.

grew normally in the soil samples that represented trees bearing healthy fruit, whereas average age values of 22 to 31 were obtained on soils representing trees bearing boron-deficient apples.

#### SUMMARY

A greenhouse procedure for determining the relative boron contents of soils has been presented. Five sunflower plants were grown in 1 pound of soil, and the criterion of the boron status of the soil was the age of the culture when the initial symptoms of boron deficiency on the sunflowers appeared. This age is referred to as the "age value." It was found to be reproducible in different aliquots of the same medium and capable of detecting an initial addition of 9 γ of boron in a pound of sand.

To compensate for certain variations in environmental conditions and handling practices, a set of quartz sand cultures to which definite increments of boron was added was included in each experiment. Age values for soils were then evaluated in relation to the quartz sand calibration series. Instead of interpreting the results of a soil in terms of age value *per se*, the soil was compared to one of the quartz sand cultures with a similar age value.

Applicability of the procedure was studied by determining the age values of a large number of soils the boron status of which was known from field experimentation. Particular attention was devoted to soils producing alfalfa, but soils from apple orchards were also included. An excellent correlation was obtained between age values of the orchard samples and boron-deficiency records on the apples.

Under the conditions of the experiments reported in this study, a deficiency of boron could be expected to exist in a field of alfalfa if the age values of soils from the 0-12- and 12-24-inch horizons average less than that of the 0.30 p.p.m. B quartz sand culture. This was approximately 36 days. Although the choice of this limit may vary, the degree of variation depending on a number of conditions, a significant feature is the parallelism between the range of boron contents or degree of deficiency in the field and the age values of the corresponding soils as determined by this procedure.

#### REFERENCES

- (1) COLWELL, W. E., AND BAKER, G. O. 1939 Studies of boron deficiency in Idaho soils. *Jour. Amer. Soc. Agron.* 31: 503-512.
- (2) COLWELL, W. E. 1943 A biological method for determining the relative boron contents of soils. *Soil Sci.* 56: 71-94.
- (3) SCOFIELD, C. S., WILCOX, L. V., AND BLAIR, G. Y. 1940 Boron absorption by sunflower seedlings. *Jour. Agr. Res.* 61: 41-56.
- (4) STEPHENSON, R. E., AND SCHUSTER, C. E. 1941 Laboratory, greenhouse, and field methods of studying fertiliser needs of orchard soils. *Soil Sci.* 52: 137-153.



## MODIFIED MITSCHERLICH METHOD FOR SOIL CULTURES

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The Experiment Station of the Hawaiian Sugar Planters' Association has used a modified Mitscherlich method for testing sugar cane soils for their nutrient availability since 1930, and at the end of 1941 had tested more than 3,600 soil samples by this method. Many of these soils were taken from plots of field experiments; others came from fields in which previously conducted experiments had already indicated the optimum amounts of plant foods needed for maximum sugar yields. Thus it has been possible to find the relationships between the results from the modified Mitscherlich testing and the actual fertilizer needs as determined by reliable field experiments, and to establish reliable and critical levels for qualitative groupings of the results from the Mitscherlich tests, and then to give to these groupings a semiquantitative interpretation designating a most probable amount of a specific nutrient to be supplied in the fertilizer. It is largely due to these facts that this method of testing soils has given reliable guidance.

The Mitscherlich test uses a plant as the analyst. The criterion is the ratio of the dry weight produced by an indicator crop grown on the soil when a specific nutrient was omitted from its fertilizer, to a corresponding dry weight produced when this specific nutrient had been adequately supplied. Thus the amount of the actual yield made by the indicator crop when any one nutrient only has been omitted must be due to the available amount of that plant food in the soil; and it will be shown later how this can be converted numerically to pounds per acre.

The principal emphasis in this test is laid on the diagnosis of the supplies of available phosphate and potash. The soil nitrogen is so fugitive that its estimation has only limited value; the supplies of phosphate and potash are more stable.

The method has several distinct advantages. First, it is highly reliable in its ability to identify a soil that has a fully adequate amount of either phosphate or potash, and so it will forestall any needless fertilizer expenditures for these two plant foods. Second, it will identify phosphate and potash deficiencies in a soil and thus afford assurance that there will be a response to fertilizer carrying one or both of these nutrients if the fertilizer is properly used. Third, and this point has real significance if results are to find practical application, it has a high psychological value for the planter whose soil is being tested, since it offers visual evidence of actual nutrient response, or lack of response, by a plant which is telling its own story. Thus it makes a most effective demonstration, and the Experiment Station eventually capitalizes on this fact by sending the planter a photograph of the mature crop taken just before it is harvested; this is often more convincing than the actual figures for the "estimated pounds per acre" that accompany it.

#### **SELECTION AND PREPARATION OF SOIL SAMPLES**

Within the field, at a place most nearly typical of the major part of the area, a sampling zone is chosen and, from a definitely recorded starting point in this zone, a route is laid out which covers 24 sampling points (six in each of four parallel lines) spaced about 10 paces apart each way. At each of these 24 points the loose surface litter is scraped away and soil is then taken up with a 4-inch post hole auger to a depth of 12 inches. The complete sample, which should supply not less than 120 pounds of air-dry soil, is placed in clean heavy canvas bags for transportation.

If the soil is too wet when received, it is spread out in shallow trays of an indoor drying rack and dried slowly until its moisture content is low enough to allow convenient handling. It need not be dried to a point where it is actually dusty. When sufficiently dry, it is put through a screen (10-mesh) and then thoroughly mixed. It is then ready for potting.

#### **EQUIPMENT**

Special equipment used in the Mitscherlich test consists of sheet iron, enameled (white outside, black inside), numbered Mitscherlich pots each 20 cm. deep and 20 cm. in diameter with large drainage opening in the bottom that is covered with a metal disk to hold the soil in place. With each pot there are also a metal stand and drainage pan, and a wire support to hold plants erect. Twelve of these special pots are used for each test. They are placed around the edge of a 51-inch diameter clinostat and each 10 clinostats are rotated at four revolutions per hour from a shaft turned by a  $\frac{1}{4}$  horsepower electric motor.

Other equipment needed is a scale for weighing the soil (capacity 20 kgm.; sensitivity 1 gm.); a scale for weighing the dry material that is harvested (capacity  $2\frac{1}{2}$  kgm.; sensitivity 0.1 gm.); three 5-gallon bottles for standardized nutrient solutions; four automatic pipettes of 10, 50, 50, and 150 cc. capacity for dispensing the nutrient solutions; a 20-quart metal mixing bucket and a large-mouthed funnel for transferring soil from bucket to pots; two planting boards each 19 cm. in diameter with wooden pegs  $2\frac{1}{2}$  cm. long, one with 40 and the other with 18 pegs, evenly spaced; gallon cans with perforated bottoms for holding the harvested plant material while it is being dried; and a large vacuum oven for rapid drying of the harvested plant material.

#### **CHOICE OF PLANTS**

Oats and rye, which were originally used for indicator plants, did not make satisfactory growth in our semitropical climate. Sugar cane itself was not satisfactory because individual plant variation was too great, and only a few plants can be grown in each pot. Of more than 20 plants tried, Sudan grass stands out as the one that can be depended on to make rapid vigorous growth and to have the least variation between duplicate pots and also be highly sensitive to variations in the available soil nutrient supply. Unfortunately, Sudan grass is sus-

ceptible to pythium root rot except when adequate phosphate is supplied, and this has made it necessary, when testing for phosphate, to use another grass, *Panicum barbinode*, which is not susceptible to pythium. Panicum grass, however, is not especially sensitive to potash deficiency and so it has been found advisable to use both of these indicator crops—Sudan grass for the potash test and panicum when testing for phosphate and nitrogen.

#### POTTING

The amount of soil to be placed in the Mitscherlich pot is determined as follows: A standard flat-bottomed pot made of copper, with the same diameter as the Mitscherlich pot and exactly 6 inches deep, is filled well above its brim with the soil to be tested, dropped two or three times from about an inch above the work bench to fill all voids, and levelled off evenly with a straightedge. This is then weighed to give the theoretical weight of 6 inches of soil. This weight is referred to hereafter as the "standard pot weight" (*SPW*) for the soil being tested. Since not all this soil will fit conveniently in the Mitscherlich pot, the amount of soil (*S*) to be put into each pot when the standard pot weight (*SPW*) is known is determined from the following table:

#### I—Undiluted or straight soil:

<i>SPW (kgm.)</i>	<i>S (kgm.)</i>	<i>SPW (kgm.)</i>	<i>S (kgm.)</i>
3.0 to 3.5	2.5	5.0 to 5.5	4.5
3.5 to 4.0	3.0	5.5 to 6.0	5.0
4.0 to 4.5	3.5	6.0 to 6.5	5.5
4.5 to 5.0	4.0	6.5 to 7.0	6.0

#### II—Diluted soil:

<i>SPW (kgm.)</i>	<i>S + Sand (kgm.)</i>	<i>SPW (kgm.)</i>	<i>S + Sand (kgm.)</i>
3.0 to 5.0	1.4 + 4.2	5.0 to 7.0	1.5 + 4.5

The required amount of straight soil, or of soil and silica sand, is weighed into a scale pan and then transferred to the mixing bucket. The required nutrients, which have been prepared in standardized solutions<sup>1</sup>, are then siphoned from their containers into automatic pipettes of 10 cc. capacity for the nitrogen solution, 50 cc. and 150 cc. for phosphoric acid, and 50 cc. for the potash solution. From these pipettes the proper nutrients are then added to the soil in the mixing bowl and thoroughly mixed in by hand. This fertilized soil is then transferred to its Mitscherlich pot.

<sup>1</sup> A filtered aqueous solution of ammonium nitrate is adjusted to a concentration of 1.1 gm. of nitrogen in each 10 cc.

For phosphoric acid, treble superphosphate is dissolved in water with the aid of compressed air bubbled into the solution. After settling, the clear supernatant solution is siphoned off into another container and diluted to carry 3.0 gm. of  $P_2O_5$  in 50 cc. of solution.

The potash solution is prepared from sulfate of potash dissolved in water, filtered, and adjusted to contain 1.5 gm. of  $K_2O$  in each 50 cc.

Tap water is used in making and diluting these solutions, since the materials are all of ordinary fertilizer grade.

Twelve pots, which comprise the standard test, are prepared according to the following plan:

NUMBER OF POTS	TO BE MARKED	TO BE PLANTED WITH	TO BE FILLED WITH	FERTILIZER SOLUTION TO BE ADDED			NUTRIENTS SUPPLIED		
				N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
2	No P <sub>2</sub> O <sub>5</sub>	Panicum	Straight soil	10	0	50	1.1	0	1.5
2	Complete	Panicum	Straight soil	10	150	50	1.1	9.0	1.5
2	No N	Panicum	Straight soil	0	150	50	0	9.0	1.5
3*	No K <sub>2</sub> O	Sudan	Soil + Sand	10	50	0	1.1	3.0	0
3*	Complete	Sudan	Soil + Sand	10	50	50	1.1	3.0	1.5

\* Since the variation in growth on the soil + sand mixture is slightly greater than on the straight soil, three pots give more reliable average dry weights than two pots.

#### PLANTING

After the 12 pots have been filled and transferred to their positions on the clinostats in the greenhouse, they are ready for planting. The 18-peg planting board is pressed into the surface of the straight soil in six pots, and into each hole thus made, a single-joint cutting of panicum grass (about 1 inch long), which has been pregerminated in a bed of pure silica sand, is planted in a vertical position just below the surface. The 40-peg planting board is used to make holes in the surface of the diluted soil in the other six pots, and into each of these holes two or three Sudan grass seeds are dropped and covered. Later, when germination can be clearly seen, a stand of only 40 of these Sudan grass plants is left in each pot.

#### CARE OF PLANTS

Immediately after planting, all pots receive a light irrigation, which is repeated and increased as needed. After the plants are well started, they receive ample tap water (from a hose), and any leachate caught in the drainage pans is returned with the subsequent irrigation so that no nutrient is ever lost from the soil by leaching. Weighing the pots to replace water used has been found unnecessary.

While the crop is growing, attention to prevent occasional insect injury may be necessary and, on very alkaline soils, a spraying with iron sulfate to prevent chlorosis is often needed. Otherwise the plants need little attention beyond irrigation.

#### HARVESTING

The test is ready to harvest when the completely fertilized Sudan grass plants have matured their seed and before it shatters; this will be about 70 to 90 days after planting. The stems are cut off at the surface of the soil; then both stems and leaves are cut into short sections (about 6 inches long) to fit them into the cans in which they are to be dried. Drying takes place overnight in a vacuum oven, or after several days in a predrier with a few hours in the vacuum oven.

## BASIC RATIOS

From the average dry weights of the crops harvested from the different treatments are calculated the basic ratios for reference to the Mitscherlich tables and estimation of the availability of the nutrients in the soil tested. After these average dry weights are obtained, the following calculations are made: (a) the "per cent of the maximum" for no  $P_2O_5$ , (b) the "per cent of the maximum" for no  $K_2O$ , (c) the "per cent increase for N" over no N. These percentage figures

TABLE 1  
*Mitscherlich's table of yields for phosphoric acid and potash\**

PER CENT OF THE MAXIMUM FOR $P_2O_5$	PER CENT OF THE MAXIMUM FOR $K_2O$	UNCORRECTED LBS. PER ACRE IN SOIL TESTED		PER CENT OF THE MAXIMUM ACRE IN SOIL TESTED	UNCORRECTED LBS. PER ACRE IN SOIL TESTED		PER CENT OF THE MAXIMUM FOR $P_2O_5$	UNCORRECTED LBS. PER ACRE IN SOIL TESTED		PER CENT OF THE MAXIMUM FOR $K_2O$	UNCORRECTED LBS. PER ACRE IN SOIL TESTED
		For $P_2O_5$	For $K_2O$		For $P_2O_5$	For $K_2O$		For $P_2O_5$	For $K_2O$		
1.5	2.3	1	27.7	39.5	21	47.8	63.5	42	84.4	94.4	120
3.0	4.6	2	28.9	41.1	22	49.4	65.2	44	86.6	95.6	130
4.6	7.0	3	30.0	42.4	23	51.0	66.9	46	88.5	96.5	140
6.0	9.2	4	31.0	43.8	24	52.4	68.4	48	90.2	97.3	150
7.4	11.3	5	32.1	45.1	25	53.9	69.9	50	91.6	97.8	160
8.8	13.4	6	33.1	46.4	26	56.0	72.0	53	93.8	98.7	180
10.2	15.4	7	34.2	47.7	27	58.0	73.9	56	95.5	99.2	200
11.7	17.5	8	35.2	48.9	28	59.9	75.7	59	96.7	99.5	220
13.0	19.4	9	36.2	50.1	29	61.7	77.4	62	97.6	99.7	240
14.3	21.3	10	37.1	51.3	30	63.5	79.0	65	98.2	99.8	260
15.6	23.1	11	38.1	52.4	31	65.6	80.9	69	99.0	99.9	300
17.0	25.1	12	39.1	53.6	32	67.7	82.6	73	99.6	100.0	350
18.3	26.9	13	40.0	54.7	33	69.6	84.2	77	99.8	100.0	400
19.5	28.5	14	40.9	55.8	34	71.5	85.7	81	99.9	100.0	450
20.7	30.2	15	41.8	56.8	35	73.2	87.0	85	100.0	100.0	500
21.9	31.8	16	42.8	57.9	36	75.2	88.5	90			
23.2	33.6	17	43.6	58.9	37	77.0	89.8	95			
24.3	35.1	18	44.5	59.8	38	78.7	90.9	100			
25.5	36.6	19	45.3	60.8	39	80.3	92.0	105			
26.6	38.1	20	46.1	61.7	40	81.8	92.9	110			

\* Computed by D. A. Cooke from Mitscherlich's "Table of Yields." To use this table: Enter at the nearest figure for "per cent of the maximum" and find the corresponding equivalent in pounds per acre on the same line. This uncorrected amount when multiplied by the "acre-foot soil factor" gives the estimated pounds of the nutrient per acre-foot of soil.

are then referred to table 1 or 2 where a related figure for "uncorrected pounds per acre" is found. This latter figure is then multiplied by a specific "acre-foot soil factor" which has been found by the following formula: acre-foot soil factor

$$= \frac{SPW \times 2}{S}, \text{ in which } SPW \text{ is the standard pot weight of soil in kilograms, } 2 \text{ is a constant showing the relation between the 6-inch depth of the standard pot and the depth of an acre of soil 1 foot deep, and } S \text{ is the amount of soil in kilograms placed in the Mitscherlich pot for testing. Perhaps right here, an example to}$$



*Fig. 1. Upper left:* Newly planted and nearly mature indicator crops growing in Mitscherlich pots arranged on clinostats in greenhouse.

*Upper right:* The stand, pot, drainage hole cover and pan, and the complete Mitscherlich pot ensemble with wire plant support.

*Center right:* Nutrient solution bottles, automatic pipettes, soil bucket, scale, standard pot, mixing bowl, and planting boards.

*Lower:* The 12 pots of a Mitscherlich test photographed just before harvest.



illustrate this procedure is in order. The lower photograph in figure 1, of the 12 pots just before harvest, shows the plants from which the following example was taken:

**Example.** Given the following soil weights:

1—Standard pot weight,  $SPW = 5.75 \text{ kgm}$ .

2—Weight of straight soil actually used in pot,  $S = 5.0 \text{ kgm}$ .

3—Weight of soil used in the soil + sand mixture,  $S = 1.5 \text{ kgm}$ .

The acre-foot soil factors would then be

$$\text{For straight soil } \frac{5.75 \times 2}{5.0} = 2.30$$

$$\text{For the soil + sand mixture } \frac{5.75 \times 2}{1.5} = 7.67$$

Also given the following average dry weights harvested:

1—Of panicum from the no  $P_2O_5$  pots ..... 216.7 gm.

2—Of panicum from the complete pots ..... 279.2 gm.

3—Of panicum from the no N pots ..... 18.6 gm.

4—Of Sudan grass from the no  $K_2O$  pots ..... 61.2 gm.

5—Of Sudan grass from the complete pots ..... 124.0 gm.

Thus the "per cent of the maximum" for no  $P_2O_5$  would be  $\frac{216.7 \times 100}{279.2}$  or 77.6, and reference to table 1 shows that this percentage figure, which lies between 77.0 and 78.7, corresponds to 97 pounds of  $P_2O_5$ . Hence 97 pounds multiplied by 2.30 (the proper acre-foot soil factor) equals 223 pounds of  $P_2O_5$ , which is the estimated amount of available phosphate in an acre of this soil 1 foot deep.

The "per cent increase for N" would be  $\frac{100(279.2 - 18.6)}{18.6}$  or 1,401, and reference to table 2 shows that this is equivalent to 14 pounds of nitrogen. When this is corrected, that is, multiplied by 2.30, we have an estimated amount of nitrogen of 32 pounds per acre foot.

Similarly the "per cent of the maximum" for  $K_2O$  in this example is  $\frac{61.2 \times 100}{124.0}$  or 49.4 and from table 1 this corresponds to 28 pounds; when this is multiplied by its proper acre-foot soil factor (7.67) the available potash is estimated at 215 pounds per acre-foot of soil.

Thus by obtaining the basic ratios of the dry weights grown without the principal nutrients to the dry weights grown with them, and by referring these ratios to the Mitscherlich tables and using a specific factor which is concerned with the specific volume weight of each soil potted, a quantitative estimate is obtained of the amount of each nutrient that was available to the indicator crop during its growing period. The next step is to see that these amounts are correctly interpreted and made useful to the planter.

#### INTERPRETATIONS

Because of the many Mitscherlich tests on soils taken from actual field experiments in which optimum amounts of plant foods were being determined, it has been possible to make many studies between the results from these two methods of soil testing. Furthermore, in perfecting the improved Mitscherlich technique, much has been learned about the limitations of this test. And there are many

things about it which are quite empirical and which, if changed, are likely to influence the basic ratios upon which the correct estimates of available nutrients are made from the Mitscherlich tables. Thus, different indicator plants will show differences in the basic ratios ("per cent of the maximum" and "per cent increase") even though grown on the same soil. Seasonal effects during the growing period will influence these basic ratios; the ratios from the same soil will not always be the same for a test made in summer as in winter. Changing the proportions of soil and sand for the potash test has had inconsistent effects on the "per cent of the maximum" on different soils, sometimes increasing and sometimes decreasing this figure as the dilution was increased. When testing for phosphate, an extension of the growing period to 30, 60, and 90 days beyond the

TABLE 2

*Pounds of nitrogen per acre as indicated by a per cent increase in yield for 1.1 gm. N over the yield obtained without addition of nitrogen fertilizer\**

PER CENT INCREASE FOR N	UNCORRECTED LBS. PER ACRE	PER CENT INCREASE FOR N	UNCORRECTED LBS. PER ACRE	PER CENT INCREASE FOR N	UNCORRECTED LBS. PER ACRE
9051	2	561	34	153	110
4872	4	499	38	138	120
3334	6	449	42	125	130
2436	8	406	46	115	140
1974	10	373	50	105	150
1624	12	333	55	97	160
1407	14	305	60	89	170
1218	16	278	65	83	180
1092	18	257	70	77	190
974	20	237	75	72	200
880	22	222	80	63	220
812	24	206	85	56	240
745	26	194	90	50	260
689	28	182	95	45	280
640	30	171	100	40	300

\* Computed by D. A. Cooke from Mitscherlich's "Table of Yields." To use this table: Enter at the nearest figure for "per cent increase for N" and then follow directions given in footnote to table 1.

initial harvest (at 87 to 92 days) has changed the "per cent of the maximum" figure from an average of 55.3 to 63.7, 68.4, and 74.5 respectively. Varying the amount of N applied per pot between 0.7 and 1.1 gm. when testing for potash has caused a variation in the "per cent of the maximum" figure between 79.2 and 94.8. When the amount of P<sub>2</sub>O<sub>5</sub> used in the "complete" pots to produce the so-called maximum was increased, the "per cent of the maximum" for no P<sub>2</sub>O<sub>5</sub> decreased, but not to the same degree with different soils. Although 1.5 gm. of K<sub>2</sub>O is needed to produce the maximum yield on most soils, it is too much and will depress growth on others that have an initially high salt content; this results in a basic ratio of more than 100 per cent for no K<sub>2</sub>O on such soils. All of these influences must be recognized, and because of them it is necessary to set up and

follow a standard procedure and learn how to interpret its results, if this method of testing soils is to be used as a substitute for the replicated field experiment.

The establishing of qualitative groupings of the results from these tests has already been referred to. Unfortunately, we cannot subscribe to the belief that the Mitscherlich method "enables us to calculate with exactness how much of a lacking element must be applied per acre, and how much yield can be obtained from the application of this or that amount of fertilizer under the existing conditions."<sup>2</sup> Perhaps this utopianism might be attained in culture solutions or in pure sand, but in soils there are too many factors which can influence the fertilizer after it is applied and which limit its complete action on growth.

The table of qualitative groupings of results from the modified Mitscherlich test is as follows:

Estimated pounds per acre foot

GROUP	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N
LOW	<60	<350	<100
DOUBTFUL	60-100	350-550	100-150
MEDIUM	100-120	550-750	150-200
HIGH	>120	>750	>200

This table is used as follows: (a) When the Mitscherlich test has indicated that the P<sub>2</sub>O<sub>5</sub> is in the HIGH group, one may be confident that there will be no response to phosphate and this nutrient can be safely omitted from the fertilizer for sugar cane. The same confidence and conclusion apply when the K<sub>2</sub>O falls in the HIGH group. And with nitrogen in this HIGH grouping, no immediate application of nitrogen fertilizer is necessary. (b) When the LOW grouping is indicated for these plant foods, one may be quite confident that sugar cane will respond if the indicated deficiency is corrected by the proper fertilizer. The actual amount, however, that should be applied is more difficult to determine, and experience has shown that it is unwise to attempt to obtain this amount from the actual figures calculated from the Mitscherlich tables, for these figures are based on an assumed constancy for the so-called "effect factors," which evidence does not wholly support. Moreover, it is believed that quantitative exactness in connection with the determination of soil nutrient content, which may be theoretically possible, is actually unobtainable for field soils, largely because the taking of soil samples from the field is not a sufficiently exact science to warrant mathematical exactness in the recommendations that must be made from the results of the analysis of the sample. Hence the aim is approximations, and guidance comes from the relationships already established between the Mitscherlich tests and the field experiments. Thus it is known that soils from the high-rainfall areas which fall into the LOW potash group will respond to 250 pounds K<sub>2</sub>O per acre, whereas those from lower-rainfall areas in this same LOW

<sup>2</sup> From translation by Chas. Richter of "E. A. Mitscherlich's Doctrine on the Determination of Fertilizer Requirements of Soil" by B. Marquart (1925) in the Hawaiian Sugar Planters' Association Experiment Station Project File 95.3.

potash group will seldom show a response to more than 175 pounds. Only experience can supply such guidance.

Actual requirements for soils that fall into the DOUBTFUL and MEDIUM groupings are even more difficult to determine. As a general rule, the critical levels appear to be at 100 pounds for P<sub>2</sub>O<sub>5</sub> and at 550 pounds for K<sub>2</sub>O; that is, there are but few instances where actual response has been obtained in the field when the modified Mitscherlich test has shown an amount above these levels. Unless there is other reason to suspect a possible deficiency, therefore, soils which fall into the MEDIUM group can generally be handled like those in the HIGH group. And soils falling in the DOUBTFUL group are usually supplied with about 100 pounds per acre of their deficient nutrient as insurance against too great an inadequacy.

Inability to make use of the actual amounts specified in the Mitscherlich tables in making recommendations of amounts to be applied in the field has not greatly detracted from the usefulness of the method itself. After its relationships with reliable field experiments have been found, it becomes a most useful tool for extending the application of the field test results to the much greater areas not included in the experiments, but like any other tool or measuring device it must first be carefully calibrated.

#### SUMMARY

The modified Mitscherlich method for testing soils for plant needs, more especially for phosphate and potash, has found extensive usage with sugar cane soils in Hawaii.

Several changes in the original method have been made. These have been chiefly concerned with the indicator crop, the soil dilution, the amount of phosphate supplied to ensure maximum growth, and the use of a specific weight of soil in each pot.

The actual procedure currently being used has been described, and a complete outline of the treatments used in the complete standard test has been given.

The method of calculating an "acre-foot soil factor," which is later used in correcting the amounts found in the Mitscherlich tables, is shown and an example giving the calculations involved in finding the basic ratios for reference to the Mitscherlich tables is explained.

A discussion is included to show the nature of certain factors that can influence these basic ratios.

A table of qualitative groupings for the results from this method of testing soils precedes a discussion of how the results are interpreted when recommendations are made for the fertilization of the cane areas from which the soil samples were taken.

## MODIFIED NEUBAUER METHOD FOR SOIL CULTURES

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For many years the two extremes, field experiments and soil analyses, represented the principal methods employed in control and experimental research in soil fertility. It is true that during this period pot experiments were frequently used, but little quantitative value was attached to them preceding the introduction of the Mitscherlich method. The growing plant has a distinct appeal in soil fertility studies, and though the Mitscherlich method reduces the gap between soil analysis and the field experiment it requires a large outlay of equipment and is a time-consuming procedure of no small magnitude. For this reason the technic employed in the Neubauer test fills a long-felt need and it is now widely employed in the routine examination of soils. Since 15- to 18-day-old seedlings and only 100 gm. of soil are required, the method has a distinct advantage over the Mitscherlich method or other pot tests which require large volumes of soil and in which the plants are grown to maturity. The Neubauer test was originally proposed as a quantitative method for the determination of available potassium and phosphate in soils but has now been developed into a research tool widely applicable to soil problems (2, 4, 6).

### THE NEUBAUER METHOD

The technic of the method, as recommended by Neubauer, is as follows:

Mix 100 gm. of air-dry soil, which has been ground to pass a 2-mm. sieve, with 50 gm. of nutrient-free quartz sand and spread evenly over the bottom of a glass dish having a diameter of 11 cm. and a depth of 7 cm. Over this layer spread 250 gm. of quartz sand. To facilitate watering, place a small glass tube upright in the center of the dish. In the surface of the sand layer, carefully plant 100 selected and weighed rye seed which have been treated with "Semesan Jr." dust disinfectant. Add 80 gm. of distilled water and keep the cultures at a temperature of 20°C., avoiding exposure to direct sunlight. As a blank test, grow plants under similar conditions in quartz sand alone. Harvest the plants after 17 days. Carefully wash them to free them of adhering soil; then dry, ash them at 550°C., take the ash up in HCl, and make it up to volume in a volumetric flask. Determine potassium and phosphate in aliquots of this.

The instructions emphasize close adherence to the recommended procedure, especially temperature, growing period, quality of rye seed, kind of container for growing the seedlings, basic values for interpreting the data, and method of adding water to the cultures. Since many institutions have limited equipment and facilities, the method, like any other new departure in soil research, has been subjected to rather critical study with reference to certain modifications in technic. Undoubtedly, this has contributed to a wider application of the test to fertility problems. In many of these modifications, attention has been given to the interpretation of the values for crops other than rye and to the substitution of seedlings other than rye as test plants. During extensive

research in plant nutrition for semiarid soils at the Arizona Agricultural Experiment Station, most of the aforementioned factors have been studied. As a result of these studies several modifications in the original Neubauer procedure have been adopted, but only after comparison of the values obtained by the two procedures.

#### MODIFICATIONS STUDIED

##### *Kind of container*

A glass dish 11 cm. in diameter and 7 cm. in depth is recommended as the culture vessel for the Neubauer test. This requires an extensive outlay of glassware when 100 or more vessels are in use at one time, and the loss from breakage is appreciable. After a study of other containers, enamelware pans were chosen with 14 cm. top diameter, tapering off to 9 cm. bottom diameter, and 7 cm. deep. The results obtained with such containers have proved entirely satisfactory.

##### *Quality of rye seed*

The quality of seed was found to be extremely important. Experiments with ordinary run of rye seed gave widely divergent values. When, however, registered Rosen rye seed from Farm Bureau Services Inc., Lansing, Mich., was used, no advantage was found to accrue from weighing each lot of 100 seeds used for the cultures.

##### *Method of planting seed*

The original Neubauer method suggests that the seed be planted in the surface of the sand as the final step just before watering the culture. This has been modified as follows: The mixture of soil (100 g.) and sand (50 g.) is placed in the bottom of the dish. One hundred fifty grams of sand is spread evenly over this, 100 rye seeds are spread evenly over this surface, the whole is placed on a torsion balance, and approximately 50 gm. of sand is spread evenly over the seed. This last weight of sand is designated as approximate because all cultures, dish plus contents, are brought to the same final or total weight. This facilitates the maintenance of proper moisture content in the cultures during the growth of the seedlings as the water is added to the proper weight.

##### *Addition of water*

The Neubauer method suggests the use of a glass tube in the center of the dish to facilitate watering. Many clay soils are heavy and badly dispersed and do not take water readily by this method. The method of watering has been modified by using a narrow-mouthed bottle fitted with a cork and sprinkler by means of which the water is sprinkled evenly over the surface of the culture until the culture dish and contents are brought to the proper weight on the torsion balance. By this procedure all the soluble salts and nutrients in the soil, which may be drawn to the surface of the sand by evaporation, will be washed back into

the sand-soil mixture in the bottom of the pan where most of the roots are gathered.

#### *Light*

Neubauer found little or no difference between values obtained by growing the seedlings in light and in dark. In growing the plants in the open room or greenhouse no attempt is made to control light, except that direct sunlight is avoided. When the plants were grown in a Minnesota seed germinator the plants lodged badly because of the tendency of the seedlings to lean toward the light entering the glass doors of the germinator.

#### *Temperature*

Temperature is one factor which is strongly stressed by Neubauer and also by Thornton. Both advise a temperature of  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . In our Neubauer studies attempts to grow rye seedlings in a Minnesota seed germinator, equipped with thermostatic control and maintained at  $20^{\circ}\text{C}$ ., failed because of the excessive seedling loss from "damping off." Also the seedlings grown in the germinator were notably less turgid than those grown under room conditions. In view of this, it became necessary to study seedling growth at room and greenhouse temperatures, which are close to  $22^{\circ}\text{C}$ . from October to April. During the summer months the temperatures are too high for the growth of rye seedlings. The values obtained by this slight modification in temperature conditions have proved entirely satisfactory as shown by the vigorous growth, by the turgor of the seedlings, and by a comparison of the values obtained by this method and by other workers using the same soils in cultures were the temperature is controlled at  $20^{\circ}\text{C}$ . The principal difference between plants grown in the seed germinator chamber and in the room or greenhouse is that the latter must be watered more often, but this apparently does not affect the values.

#### *Growing period*

Experiments were conducted to determine the potassium and phosphate values obtained by varying the growing period from 12 to 18 days. The highest values were obtained with the 14- and 16-day-old seedlings, and on this basis a 15-day period of growth was selected from seedlings grown under the modified conditions necessitated by our limited facilities.

#### THE MODIFIED METHOD

The following modified method has been used at the Arizona Experiment Station, with complete satisfaction, for over 10 years. Mix 100 gm. of air-dry soil, which has been ground to pass a 2-mm. sieve, with 50 gm. of nutrient free silica sand and spread the mixture uniformly over the bottom of an enamelware pan 7 cm. deep and with 14 cm. top diameter tapering off to 9 cm. bottom diameter. Over this, spread 150 gm. of silica sand, on the surface of which carefully spread 100 selected, but not weighed, Rosen rye seeds which have been previously treated with "Semesan Jr." dust. Place the pan and contents on a torsion

balance and add approximately 50 gm. of silica sand or just enough to cover the seed and attain a weight which will be the same for all the cultures being grown at one time. Sprinkle 80 gm. of distilled water evenly over the surface and keep at room temperature, close to 22°C., for 15 days. The weights of the cultures are checked every 2 days with the torsion balance, and distilled water is added to bring each to the proper weight. The blank test with silica sand is conducted in the same way except that 100 gm. of additional sand is added to replace the 100 gm. of soil so that the total weight of the sand cultures will be the same as that of the soil cultures and thus facilitate watering. On harvesting, the plants are washed free of adhering soil under the tap and over a 2-mm. sieve so that none of the plant material will be lost during the washing operation. The plants are ashed at 550°C., the ash is taken up in HCl, made to volume, and analyses we made on aliquots of this solution.

During the 10 years this method has been in use at the Arizona Experiment Station comparisons have been made with chemical methods used for the determination of available potassium and phosphate in soils. The most satisfactory chemical method for calcareous soils is one in which carbonic acid is used as the extractant. Values obtained with this chemical method and the modified Neubauer correlate very closely (1, 2, 4). Also as already mentioned, in an exchange of soils close agreement between the regular and modified Neubauer method was obtained. It appears then that slight modifications in the Neubauer procedure, even to temperature, do not invalidate the method.

#### APPLICATION OF THE NEUBAUER METHOD TO RESEARCH

In addition to the routine determination of available potassium and phosphate in soils, the Neubauer technic has proved useful for research in soil fertility and fertilizer usage. Thornton (6) has used it in studies on fixation, comparison of availability of different fertilizer materials, and influence of various factors on plant food availability in soils. At the Arizona Experiment Station the modified method has been used to study the efficiency of various phosphate fertilizers on calcareous soils, and the influence of pH, CaCO<sub>3</sub>, different forms of nitrogen, magnesium, and other factors on the uptake of potassium, phosphate, and calcium (2, 4). During the course of these experiments there was some evidence that the value of the Neubauer technic might be extended by varying the weight of soil and the number of seedlings.

#### *Variation in Weight of Soil and Number of Seedlings*

In the experiments leading up to the adoption of the modified procedure just outlined, no attention was given to varying the weight of soil, number of seedlings per culture, or ratio of weight of soil to number of seedlings. In the chemical analysis of soils a large number of methods are in use. These yield widely variable values which are still further varied by slight changes in concentration of the extracting solution and ratio of weight of soil to volume of extractant. Chemical methods have thus come to be known as empirical methods. Since Neubauer method specifies a 100:100 ratio of soil to seedlings, the question

naturally arises as to the effect of varying this ratio on the value of the method for determining the available nutrients in soils and whether such a modification in procedure might broaden the usefulness of the method in soil fertility research.

In the Neubauer method the assumption is made that 100 rye seedlings will practically exhaust the readily available potassium and phosphate in 100 gm. of soil. This is not borne out by our study of soil:seedling ratios and variation in absolute weights of soil and number of seedlings per culture (4,5). For example, it was found, on a per plant basis, that a greater uptake of phosphate is attained by growing a lesser number of seedlings on smaller weights of soil, that is, by maintaining the 1:1 soil-seedling ratio but reducing it below 100:100. Furthermore, 25 seedlings will take up more potassium and phosphate, on a per plant basis, from 100 gm. of soil than will 100 seedlings. When the number of seedlings is increased to 200 per 100 gm. of soil, the per plant uptake is still further reduced. When the number of seedlings is maintained constant at 100 and the weight of soil per culture is varied from 10 to 200 g., there is a fairly steady increase in uptake of phosphate to 90-100 g. of soil but no further increase in uptake when the weight of soil is increased to 200 g. On the other hand, the potassium values continue to increase up to 200 g. of soil, although the increase beyond 100 g. is small. This is shown in figures 1 and 2 where mean values for eight soils are given for increasing weights of soil per 100 seedlings. Calcium values are given in figure 2 but, since these soils are calcareous and uptake of calcium by 15-day-old rye seedlings is very small, the data are not so useful as they might be for noncalcareous soils.

These experiments show that the soil-seedling ratio of 100:100 must be closely adhered to, just as must the ratio of solvent to soil in the chemical analysis. That is, no modifications in number of seedlings and weight of soil are permissible when the method is being used to determine available nutrients in soils. Experiments do clearly demonstrate, however, that modifications in seedling:soil ratio and absolute weight of soil or number of seedlings per culture offer an excellent new approach to quantitative greenhouse studies on nutritional problems in soils.

#### *Seedlings other than rye*

Much criticism has been directed toward the Neubauer method on the premise that no single plant can serve as an indicator of plant-food availability in soils for all crops and therefore pot experiments are more truly indicative. This brings up the question of whether seedlings other than rye can be grown by the Neubauer technic. Some experiments have been conducted with Arizona soils in which a number of different common crop seedlings were grown (4, 5). The principal limiting factor is unquestionably uniformity or lack of uniformity of seed. It is true that different plants have different feeding powers and plant-food requirements. Despite these facts, fair to excellent results have been obtained with other seedlings; that is, the uptake may be greater or less than that of rye but there is a surprising correlation when comparisons are made between large numbers of soils.

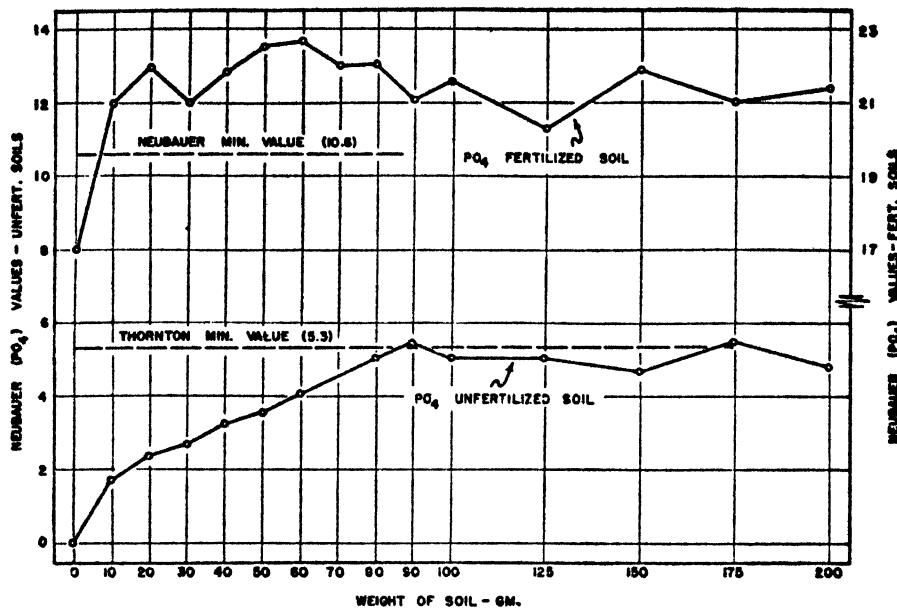


FIG. 1. EFFECT OF WEIGHT OF SOIL ON  $\text{PO}_4$  NEUBAUER VALUES FOR FERTILIZED AND UNFERTILIZED SOILS

Fertilized soils received 30 mgm.  $\text{PO}_4$ .

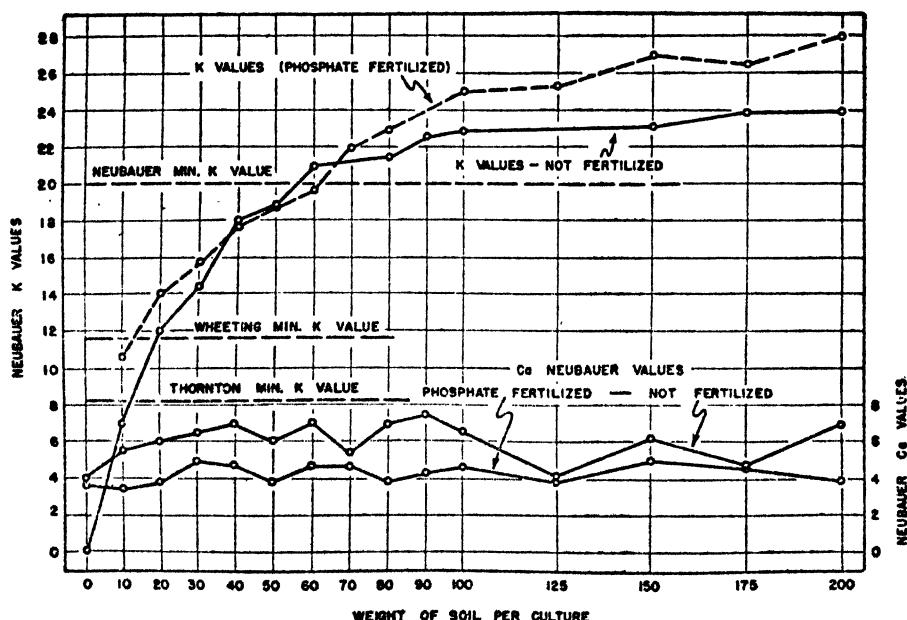


FIG. 2. EFFECT OF WEIGHT OF SOIL ON K AND CA NEUBAUER VALUES, FOR FERTILIZED AND UNFERTILIZED SOILS

Fertilized soils received 30 mgm.  $\text{PO}_4$ .

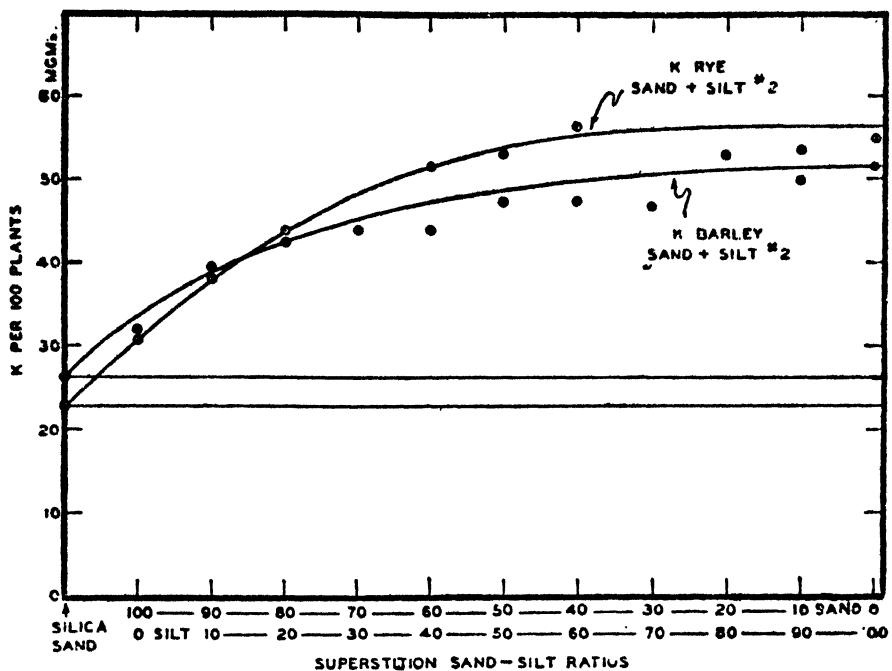


FIG. 3. MILLIGRAMS K REMOVED FROM 100 GM. SAND-SILT MIXTURES BY 100 RYE AND 100 BARLEY SEEDLINGS

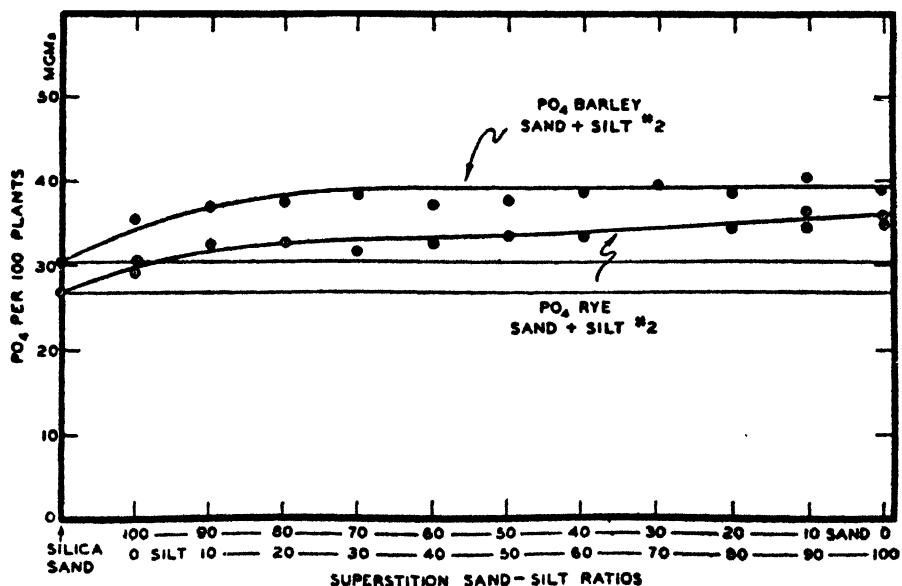


FIG. 4. MILLIGRAMS PO<sub>4</sub> REMOVED FROM 100 GM. SAND-SILT MIXTURES BY 100 RYE AND 100 BARLEY SEEDLINGS

In semiarid soils there are many growth-limiting factors such as alkali, salinity, pH, excessive amounts of  $\text{CaCO}_3$ , extreme variations in soil structure, and other factors which influence ion uptake and thus alter correlations between the rye values and those of other seedlings. The dynamic character of semiarid soils and the effect on nutrient-ion uptake should be better understood before these factors are allowed to militate against the application of the rye value to other crops. Experiments give almost positive evidence that the Neubauer method can be modified to use seedlings other than rye. Comparative values for rye and barley seedlings are shown in figures 3, 4, and 5.

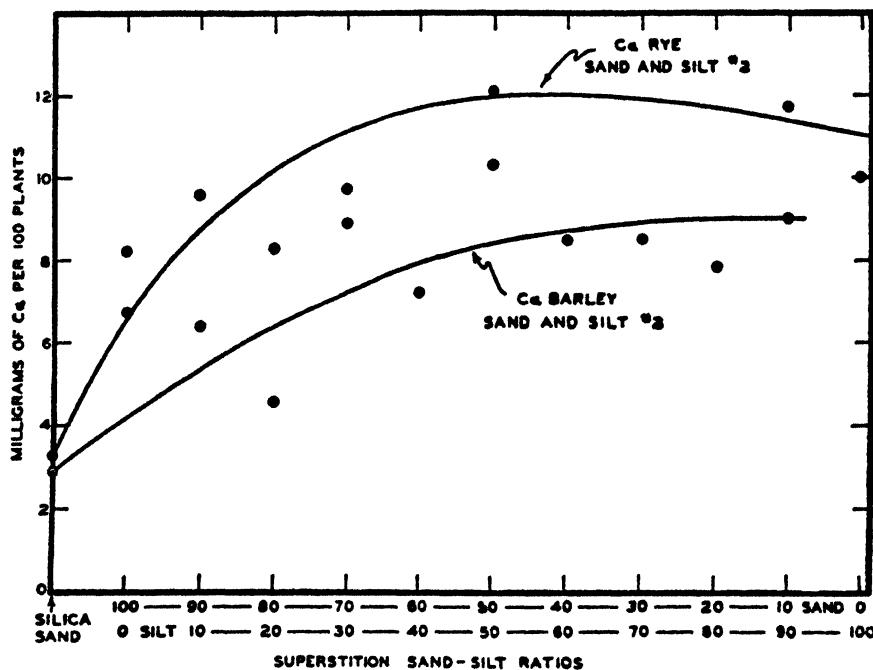


FIG. 5. MILLIGRAMS CA REMOVED FROM 100 GM. SAND-SILT MIXTURES BY 100 RYE AND 100 BARLEY SEEDLINGS

#### SOME APPLICATIONS OF THE MODIFIED NEUBAUER METHOD

To illustrate the usefulness of the Neubauer procedure in the greenhouse study of nutritional problems, several applications of the method in soil research at the Arizona Experiment Station are of interest.

The semiarid soils of the Southwest have been generally believed to be well supplied with available potassium. The Neubauer method has been used to obtain a quantitative measure of the extent of the potassium reserve in these soils (1). By reducing the weight of soil used in the test but maintaining the number of plants constant at 100, the minimum weight of soil which will yield the minimum values set by Neubauer and Thornton can be determined. This gives a clear picture of the potassium reserve as shown in figure 2. The same

procedure has been used in phosphate studies in order to make the phosphate data more nearly quantitative with respect to the available phosphate reserve.

Phosphate studies on Arizona soils have identified carbonate-phosphate as the dominant phosphate compound present in semiarid calcareous soils and have shown that this is extremely unavailable in such soil types. The Neubauer method has been used (2) to prove that similar forms of phosphate such as bone meal and raw rock have no value as phosphate fertilizers on these soils. The method has also been used to demonstrate the influence of particle size on the efficiency of less readily available phosphates such as calcined rock.

An interesting application of the Neubauer method to research was shown in a study of Yuma mesa lands for which water was recently supplied from the Colorado River (3). These lands are predominately Superstition sand, a soil which contains more than 90 per cent particles of sand dimensions or larger. It seemed advisable to use silty water rather than clear water to irrigate this land both to improve its water-holding capacity and to take advantage of the nutrient richness of the river silt. By means of the Neubauer test it was possible to show the effect of the river silt on the nutritional fertility of this sand soil. This was accomplished by mixing varying proportions of Superstition sand and air-dry silt taken from the river. These Neubauer values are given in figures 3, 4, and 5 as milligrams per 100 plants. The values are not corrected for blanks, but the blank values are represented by horizontal lines. Values are given for both rye and barley seedlings. The Neubauer test clearly proves the value that will accrue from the use of silty irrigation water on these sandy lands. The increase in available potassium is definitely greater than that of phosphate, but this is to be expected, since the silt is largely eroded material from calcareous desert lands.

#### CONCLUSIONS

Though it is advisable to adhere as closely as possible to the procedure given by Neubauer, it is believed that the method can be modified, where lack of certain facilities necessitates such modifications, without seriously affecting the interpretative value of the results.

With modifications to suit laboratory or greenhouse temperatures, preferably not over 22°C., the Neubauer method offers a procedure for the examination of the fertility of the soil in which the plant is used as an indicator. Most important is the observation that at this temperature the Neubauer method can be further modified to the extent that the method becomes an important research tool for the study of plant nutrition and especially the growth-limiting factors that are always present in greater or less degree in semiarid soils.

Like chemical soil analysis, the Neubauer method is an empirical method, and therefore adherence to the 100:100 ratio of weight of soil to number of seedlings is essential for obtaining Neubauer values. As a research tool in the study of soil and nutritional problems, it is advisable to vary this ratio or absolute weight of soil and number of seedlings in order to get a broader knowledge of the inherent soil conditions being studied. With the same technic, many seedlings

other than rye can be grown on a quantitative basis where one desires to study the growth-limiting factors for other seedlings.

#### REFERENCES

- (1) McGEORGE, W. T. 1933 Potassium in calcareous soils. Ariz. Ag. Exp. Sta. Tech. Bul. 50.
- (2) McGEORGE, W. T. 1939 Factors influencing the availability of native soil phosphate and phosphate fertilizers for Arizona soils. Ariz. Agr. Exp. Sta. Tech. Bul. 82.
- (3) McGEORGE, W. T. 1941 Influence of Colorado River silt on some properties of Yuma mesa sandy soil. Ariz. Agr. Exp. Sta. Tech. Bul. 91.
- (4) McGEORGE, W. T. 1942 Studies on plantfood availability in alkaline-calcareous soils: seedling tests and soil analysis. Ariz. Agr. Exp. Sta. Tech. Bul. 94.
- (5) McGEORGE, W. T. 1944 Some modifications in the Neubauer method. *Soil Sci.* 58: 389-397.
- (6) THORNTON, S. F. 1935 Soil and fertilizer studies by means of the Neubauer method. Ind. Agr. Exp. Sta. Bul. 399.

# LARGE-SCALE SOILLESS CULTURE FOR PLANT RESEARCH<sup>1</sup>

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Artificial or soilless cultures have been used very extensively during the last 30 years in plant nutrition studies. They have been extremely useful in many types of investigations on the physiology of plants. Within the last 15 years the artificial culture techniques of Sachs, Knop, Livingston, Shive, Hoagland, and others have been modified and expanded to permit certain new types of investigations with plants.

It is now possible, by this method, to save considerable labor when large amounts of plant material must be produced under known nutrient supply, as for example in dietary studies or for analytical purposes. Secondly, soilless cultures can be adapted to growing large plants, such as bushes and trees, under controlled nutrient supply. Furthermore, the use of large artificial cultures enables the investigator to study nutrient absorption on a large scale with a minimum of analytical detail and labor, advantages which may be secured with a possible gain, rather than a sacrifice in accuracy.

## TYPES OF LARGE ARTIFICIAL CULTURES

At the present time three types of artificial cultures are used for growing plants on a large scale: solution cultures, sometimes called "hydroponics"; sand cultures; and gravel cultures.

The first two may be considered large-scale modifications of the common solution-culture and sand-culture techniques. They have been vastly expanded by the use of mechanical equipment not utilized by their conventional small-scale forerunners. On the other hand, gravel culture, in which various kinds of cinders, gravel, or other coarse but relatively inert materials are used for root media and support, being flooded periodically with a nutrient solution applied from the bottom of the bed, has no close prototype in soilless culture.

### *Solution cultures*

The basic requirement for solution culture is a relatively shallow, water-tight tank covered by a wire screen or some other means of supporting the plants which, at the same time, allows all or a major part of their root systems to extend into the nutrient solution. The size of the tank required depends on the nature of the investigation and the root systems of the plants. In general, tanks measuring 25 by 10 feet and 6 to 10 inches in depth have proved convenient for many types of investigations. Some workers, however, prefer long, narrow tanks that permit the growth of a single row of plants.

Tanks providing more than 25 square feet of top surface should not exceed a width of 4 feet, although the length may be 50 or more feet. Concrete tanks

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more than 20 or 25 feet long should be constructed in units of not more than 20 feet and connected by U-shaped copper or zinc expansion joints.

Tanks may be constructed of wood, covered on the inside with asphalt-impregnated roofing felt. Final waterproofing is accomplished with a coating of hot, oxidized asphalt, or of paint made by dissolving oxidized asphalt in naphtha or a similar completely volatile mineral solvent.

Steel or black iron tanks are very serviceable. Such tanks made of 12-gauge sheet metal are relatively light and may be moved readily without danger of producing leaks. The Lord and Burnham Company has recently started production of sectional steel benches and tanks of various widths for soilless culture. Local sheet metal workers can also make such equipment. The tanks should be thoroughly coated on the inside with asphalt. The outside should be protected with a rust-retarding, metal paint, which, in turn, may be covered with an aluminum paint. Asphalt paint is not recommended for the outer surface because of its possible damage to clothes during hot weather.

Strong, long-lasting, reinforced concrete tanks or trays may be made at a relatively low cost per unit if sufficient cultures are needed to warrant the construction of a steel form. The Portland Cement Association will furnish advice, on request, for the construction of such concrete tanks. The use of sharp cinders as aggregate will minimize the weight of concrete tanks without sacrifice of durability. This may be of considerable importance when portable units are required.

*Support for plants.* In order that the roots may be suspended in the nutrient solution, the weight of the plants must be supported in some manner, either by the larger roots at the crown, or by the tops. Wire screen, of  $\frac{1}{4}$ - to 1-inch mesh, stretched tightly across the tank about 1 to 2 inches above the surface of the nutrient solution is commonly used for this purpose. Galvanized screen is readily obtainable, and a heavy coating of asphalt will render it inert.

It is very important to exclude light from the roots as well as from the nutrient solution. Aside from any effect it may have on the growth of roots, light favors the development of algae in the nutrient solution. This should be avoided, especially when quantitative absorption studies are involved. Glass wool, covered with sphagnum, excelsior, straw, gravel, or various other nontoxic materials is satisfactory. Depending upon the nature of the plants grown, this covering may have to be about 1 to 4 inches thick to exclude light and to cover the crowns. Bulb and tuber crops, particularly, need a thick covering.

Sheet metal and wooden covers with holes cut in them for insertion of the plants have been found convenient by some investigators. The plants are secured in the covers by a variety of methods, the most common of which has been the use of split discs of cork or wood stuffed with nonabsorbent cotton.

*Circulation of nutrient solution.* Gericke (12) has described large solution culture units which do not involve mechanical circulation of the liquid. Such cultures depend upon diffusion for distribution of nutrients through the liquid. Arnon and Hoagland (5) used solution cultures in which some circulation was provided by the escape of air bubbles through a perforated pipe or through a porous carbon tube.

When the effects of a critical balance of nutrient ions are being studied, some means of circulating the solution about the roots is particularly desirable. Nightingale (17) has shown that, as a result of differential absorption and respiration, rapid changes in pH occur in the solution immediately surrounding roots. Provision for mechanical circulation of the solution is required also where large tanks are used, not only to give uniform nutrient distribution, but also to facilitate the replenishment of nutrients in the solution.

Alexander *et al.* (1) have described a simple and effective method of circulating nutrient solutions through their tanks and, at the same time, aerating the cultures. A similar arrangement is diagrammed in figure 1. A T-connection placed at one end of the tank is fitted with a nipple and petcock long enough to extend above the level of the nutrient solution. By adjusting the opening in the

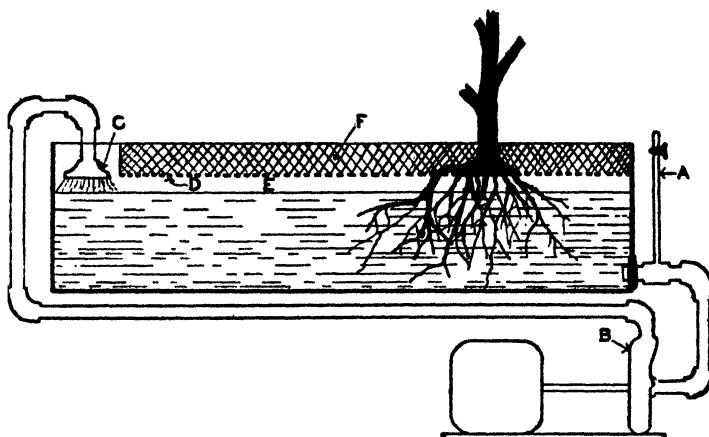


FIG. 1. SOLUTION-CULTURE UNIT WITH PROVISION FOR CIRCULATION AND AERATION OF NUTRIENT SUBSTRATE

*A*—Air intake on suction line of pump; *B*—centrifugal pump operated by motor; *C*—spray nozzle for aerating solution; *D*—wire screen support for plants; *E*—air space between wire screen and nutrient solution; and *F*—mulch over screen and roots.

petcock, one can regulate the mixture of air and nutrient solution drawn in by the centrifugal pump. A broad, round nozzle placed 2 or 3 inches above the level of the nutrient solution, but at the end opposite to that from which the solution is withdrawn by the pump, provides additional aeration. These nozzles, constructed of aluminum, are available at various horticultural supply stores. Depending upon the nature of the investigation, the amount of circulation, and the oxygen concentration required, the pump may be operated continuously or periodically. Several small centrifugal pumps may be belt-driven from a shaft and a single motor.

*Aeration.* It is a well-established fact that, unless provision is made for aeration, solution cultures seldom supply enough oxygen to roots to permit optimum growth and functioning of the plants. A space of an inch or two between the surface of the nutrient solution and the base of the plant support is an

important feature of cultures not provided with mechanical aeration. In the moisture-saturated atmosphere present in this space roots usually develop an abundance of root hairs, which in turn absorb oxygen. Except for a shallow zone near the surface of the nutrient solution, the concentration of oxygen present is too low. Allison (2) showed that marked increases in growth resulted when air was bubbled into solution cultures. Shive (20) has shown that the optimum oxygen concentration in solution cultures varies for different species. For soybeans he found the optimum to be 6 p.p.m., for oats 8 p.p.m., and for tomato plants 16 p.p.m.

Several methods for aerating large-scale solution cultures are in common use. Figure 1 illustrates one of them. Small cascades built into one end of the solution tank also have given good results. Nutrient solutions are pumped to the top of the cascades and allowed to trickle in shallow layers back into the tanks. Arnon and Hoagland (5) forced air under low pressure through porous carbon tubes lying on the bottom of their solution tanks. These tubes are manufactured by the National Carbon Company, Inc., and can be obtained in various sizes. When air is passed through the tubes under water, myriads of fine bubbles are formed. By varying the size and number of carbon tubes used, as well as the air pressure, a wide range of oxygen concentrations may be obtained.

*Temperature of nutrient solutions.* For some investigations, it is necessary to maintain the nutrient solutions at certain temperatures. Lead-covered soil-heating cables painted with asphalt have been used for this purpose, the temperature of the solution being controlled thermostatically. The solution tanks may also be equipped with pipes through which hot water is circulated. In this case solenoid valves, thermostatically controlled, may be used to maintain the desired temperature. The latter system may also be used to cool the nutrient solution when cold water is circulated through the pipes.

In general, it has been found neither necessary nor advisable to heat the nutrient solutions when the plants are grown in greenhouses maintained at a favorable air temperature.

#### *Sand cultures*

*Types of sand beds.* The same tanks, beds, or benches used for solution culture may be used for sand culture when provision is made for the removal of gravitational water or nutrient solution from the bottom. In general, however, it is advantageous to use, for large sand-culture work, beds that are deeper than the tanks required for solution culture. Thus, Thomas *et al.* (23) and Eaton (8, 9) used sand beds 18 to 30 inches deep for investigations with annuals, and Eaton used beds 6 feet deep for growing citrus trees.

The removal of excess or gravitational nutrient solution from the sand in watertight beds is facilitated by the use of one or more channels of half-round lead tile, or asphalt-coated roofing gutter. The tile or gutter pieces are placed on the bottoms of the beds, with the edges raised very slightly with thin pieces of slate or pottery, to provide for ease of distribution of the solution sidewise as well as for an unimpeded flow lengthwise. Obviously, V-shaped or otherwise

slanting bottoms are to be preferred to flat watertight beds. Moreover, a slight slant lengthwise also aids in the drainage of the sand. Usually, as little as 1 inch of slope in 50 or even 100 feet is adequate. This type of installation requires a separate nutrient-solution storage tank, and is operated in essentially the same manner as are gravel cultures. A diagram of a multiple, large-scale sand culture installation convenient for research work is shown in figure 2.

Thomas *et al.* (23) have described in detail a modification of this type of sand culture suitable for studies on root respiration, photosynthesis, and the effects of gases on plants. Eaton (8) described the construction of deep sand cultures, and recommended equipment for operating them automatically.

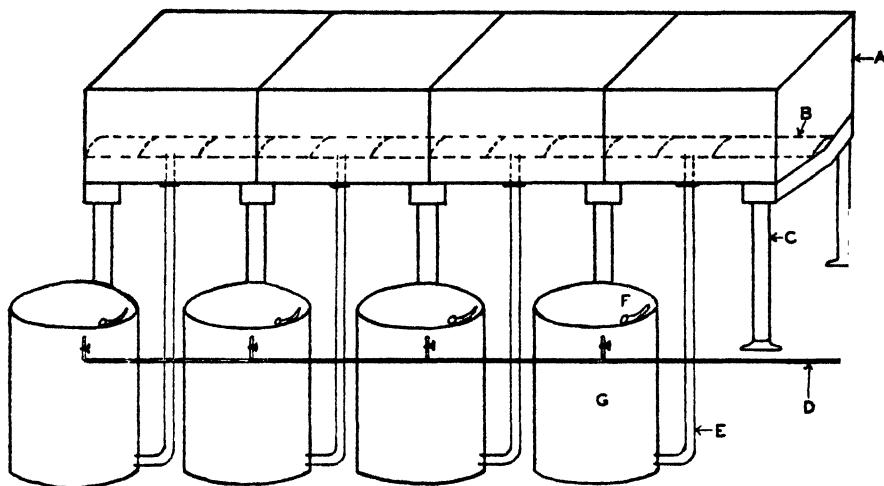


FIG. 2. MULTICOMPARTMENT SHEET-METAL RAISED BENCH SUITABLE FOR USE IN SUBIRRIGATION SAND OR GRAVEL CULTURE FOR RESEARCH WORK

A—Sheet metal; B—V-bottom bench; C—channel made of half-round lead tile; D—5-pound, compressed-air line; E—inlet and return pipe for nutrient solution; F—Quick Lox, removable drum head; and G—heavy-gauge drum available in sizes up to and including 55 gallons.

For some investigations of short duration and those for which construction costs must be minimized, ordinary greenhouse benches or curbed beds with bottom drainage may be used. In this type of sand culture, the nutrient solution is applied from the top, and the excess or displaced solution is drained through the bottom and discarded. Lath can be used to cover large cracks or spaces in raised benches, and coarse glass wool spread thinly over the narrow cracks will effectively retain the sand and allow good drainage at the same time. Ground beds curbed with boards or concrete are economically constructed if they are not already available. Adequate drainage from the bottom of the beds, and provision for limiting the vertical as well as the horizontal penetration of roots below the sand may be very important. Drainage can be provided by excavating below the floor level and replacing the soil with gravel or well-fused

cinders. A lining of heavy asphalt roofing material running lengthwise against the inside walls of the beds will prevent the lateral growth of roots outside the sand or gravel. This type of sand culture unfortunately permits the roots of plants to extend into the gravel and even into the underlying ground. The depth, coarseness, and composition of the gravel or cinder layer, together with the rooting habits of the crops used, and the duration of the experiments, therefore, are important factors to be considered with this type of sand culture.

Porous bottoms in sand beds are preferred by some investigators for studies in which the nutrient solutions are recovered and reused. Such bottoms may be constructed of concrete slabs, perforated metal, or other material, with coarse glass wool placed over the openings to retain the sand. They may also be made of porous aluminum-silicate insulating brick. Eaton (9), however, advises that not all types of such insulating brick are suitable for this purpose. Only those rapidly permeable to liquids are satisfactory. The porous bottoms thus obtained allow rapid as well as thorough drainage of the sand culture. In general, equipment of this type, because of extra construction required to collect the nutrient solutions drained through the sand, is more expensive to install than that using watertight bottoms.

*Methods of applying nutrient solutions.* Ingenious methods have been used for applying nutrient solutions to the surface of sand cultures. Eaton (8, 9) used perforated pipes through which the nutrient solutions were pumped. The pipes were placed in such a manner as to provide for good distribution of the nutrient solution over the surface of the sand. Sufficient solution was used per application to displace the residual solution from the sand. Chapman and Liebig (7), as well as Eaton (9), used compressed air to raise their nutrient solutions from reservoirs and to distribute them over the surface of the sand. Biekart and Connors (6) applied their solution to sand beds by hand through garden hose. For this method of application, the solutions may be gravity-fed, or they may be pumped. The latter method is definitely superior.

When the nutrient solutions are applied automatically to the surface of sand cultures, some investigators prefer to cover the sand with an inch or two of pea-size, or slightly larger, gravel, or with perforated ceramic-ware discs to facilitate a uniform distribution of nutrients and water. This is somewhat more important with shallow than with deep sand cultures.

Thomas *et al.* (23) applied their nutrient solutions to comparatively deep sand cultures by means of subirrigation. Eaton (8) also used the subirrigation method for deep sand beds. This method is applicable whenever watertight beds are used. For beds approximately 8 inches or less in depth, however, a distinctly coarser sand should be used when the bottoms are watertight than when they are porous. This will be considered in more detail under the discussion on quality of sands. It is a particularly important consideration where nutrient solutions are applied by the subirrigation method. When the type of construction shown in figure 3 is used, the subirrigation method of solution application may be employed with beds having porous bottoms.

*Quality of sand.* A clean quartz sand is preferred for most investigations

involving nutrition studies. Nevertheless, various relatively inert sands may be used satisfactorily for some nutrition studies, since the amounts of nutrients derived from such media may be insignificant. Lime sands often allow good growth of plants, but they are usually not desirable for nutrition studies. Likewise, sands high in reactive iron are not satisfactory for use in installations where the solutions are to be returned to reservoirs and reused.

*Size of sand particles.* Depending on the requirements of a particular investigation (that is, the plants grown, the frequency of application of nutrient solution, the amount of solution used per application, the temperature of root media, and the depth of the culture), the sand used may be medium-fine to medium-course in texture. Contrary to what might be expected, the sand used for beds 5 to 6 inches deep should be coarser than that used for beds 8 or 9 inches deep. Similarly, the sand used in beds 9 to 10 inches deep should be coarser than that used in beds exceeding 10 inches in depth. Thus, Robbins (18) found that sand ranging between approximately 0.5 and 2 mm. in diameter gave the best results with tomatoes in cultures 8 to 9 inches deep. Thomas *et al.* (23) used



FIG. 3. CROSS-SECTION OF BED WITH POROUS, FALSE BOTTOM FOR SUBIRRIGATION OF SAND OR GRAVEL

sand that was 0.5 to 0.6 mm. in diameter for beds 2.5 feet deep. Eaton (8) found that sand which passed through a 40-mesh screen but was retained by 100-mesh, approximately half of the material ranging between 0.3 and 0.4 mm., gave satisfactory results when used in beds 1.5 to 6 feet deep.

Lebedeff (16) has shown that, when a bed of sand of medium texture is flooded with water, and the gravitational water is allowed to drain off, the moisture content will decrease progressively from the bottom upward to a height of approximately 16 inches. Above this point the moisture content will remain fairly constant and very low. He also showed that when tubes 4 to 5 cm. in diameter were filled to various heights, as indicated in table 1, with sand of medium texture, covered at one end with gauze, thoroughly flooded with water, and then all gravitational water allowed to drain off, the distribution of the remaining moisture bore a fairly definite relationship to the height of the column of sand up to 40 cm. The application of his results is of considerable importance to sand culture studies. A part of his findings, therefore, are presented in table 1. It is significant to note that in columns of sand up to 20 cm., or 8 inches, the moisture content after gravitational water has drained off remains high throughout. In 30-cm. columns, however, a marked decrease occurs in the 20- to 30-cm. layer. It is evident from these data that upper layers of deep columns of sand

retain relatively little water as compared with the lower layers. The data also indicate that the lower parts of deep columns of sand retain significantly less water than do relatively shallow columns.

In applying these data to sand cultures, one must realize, however, that the drainage of gravitational water from the upper part of a deep bed of sand into the lower part may continue for many hours, depending upon the depth of the sand.

The author found that rose plants in sand cultures 6 inches deep grew distinctly better than did similar plants in cultures 12 inches deep when nutrient solutions were applied once daily. The sand used had been screened, and ranged from 20- to 50-mesh. It was found that the upper 4 to 5 inches of the 12-inch cultures soon became too dry to support active root growth in roses. Glass-wool mulches placed on the sand had no beneficial effect, but the application of a 1-inch layer

TABLE 1

*Moisture content of sand of medium texture found by Lebedeff at different levels in columns of various heights after the gravitational water had drained off*

HEIGHT OF SAND <i>cm.</i>	MOISTURE AT VARIOUS LEVELS IN SAND					
	A <i>per cent</i>	B <i>per cent</i>	C <i>per cent</i>	D <i>per cent</i>	E <i>per cent</i>	F <i>per cent</i>
100						1.9
90						2.1
80						2.0
70						2.1
60						2.5
50					2.3	2.2
40				3.0	3.5	2.2
30			12.8	7.5	7.9	6.0
20		21.5	18.5	16.4	15.1	13.7
10	22.1	20.8	21.3	18.0	14.5	15.7
0-1	20.7	20.3	20.3	18.2	15.3	15.3

of fine sand, all of which passed through a 50-mesh screen, brought about a marked improvement in the growth of plants in the 12-inch cultures.

As a general principle based on the preceding discussion, it may be stated that where frequent applications of nutrient solutions are to be used daily with sand cultures, a relatively coarse grade of sand should be used. On the other hand, with plants of similar oxygen requirements, other things being equal, the proportion of fine sand particles present in the root media, should be increased slightly as the depth of the beds is increased. Moreover, in some instances the use of relatively coarse sand in the lower part and relatively fine sand in the upper part appears warranted in some deep beds.

*Nutrient-solution storage tanks.* Where nutrient solutions are collected and reused, readily accessible storage tanks are required. They may be constructed of wood, sheet metal, or concrete. For small installations, steel drums, as shown in figure 2, are both inexpensive and convenient. They may be protected

satisfactorily from rust and reaction with the nutrient solution by at least two thorough coatings of asphalt. If large tanks are needed, concrete probably will be found most satisfactory. It is important that such tanks be of dense concrete and of monolithic construction. The inner surfaces should be coated with asphalt.

In general, the capacity of the nutrient-solution storage tanks should be at least 2 gallons for each square foot of surface at the top of the sand bed. If smaller amounts of nutrient solution than this are used with rapidly growing plants, considerable attention may be required in reconstituting the nutrient supplies and pH values.

#### *Gravel cultures*

Gravel culture, or the growing of plants in various forms of gravel bathed periodically with nutrient solutions, has become a useful research tool as well as an economical method of growing plants commercially, especially under greenhouse conditions. When used with a suitable gravel, this method provides excellent root aeration, and is well adapted to the use of automatic operating devices.

*Construction of beds or benches.* The equipment required for large-scale gravel culture is essentially the same as that used for sand culture to which nutrient solutions are applied by subirrigation. Although the beds or benches used for the former seldom are more than 6 to 8 inches deep, there appears to be no fundamental limitation to their depth, provided a suitable type of aggregate is used. Indeed it seems likely that for growing large bushes, trees, or other plants by this method, gravel beds more than 8 inches deep should provide more uniformly favorable root environments than shallower ones. In this connection, however, it is important to use porous root media such as Haydite or cinders in deep beds, or to use a pumping cycle which will avoid excessive drying of the upper portion of the beds and still permit adequate aeration at the bottom.

Figure 2 shows, diagrammatically, gravel-culture equipment suitable for many types of research. Figure 4 illustrates a type of raised bench construction adaptable both to large-scale investigations and to commercial production.

Raised benches may be constructed of wood, metal, or concrete. In all cases, particular attention should be given to the provision of adequate support for the benches. Benches filled with gravel and nutrient solution are considerably heavier than those filled with a corresponding volume of soil. Kiplinger and Laurie (15) have described the construction of wooden and concrete benches especially designed for gravel culture. Where concrete benches are to be constructed they should be made monolithic whenever possible. The individual units in this case should not be more than about 20 feet long. Single beds much over 20 feet long should be made up of two or more units connected by V- or accordian-shaped copper or zinc expansion joints set in place when the concrete is poured.

Heavy-gauge sheet-metal bench tops and beds with V-shaped bottoms, as mentioned previously, may be purchased in a variety of widths and lengths.

These benches are convenient and flexible in their adaptation to various types of investigations.

Benches may be partitioned with heavy sheet metal, wood, transite, or concrete. Temporary partitions can be made water-tight by the use of elastic caulking compounds. After the caulking has set, it should be covered with asphalt.

If ground beds are preferred (and they are more convenient for growing tall crops than are raised benches), forms for the construction of Winandy type reinforced concrete ones with V-bottoms may be rented from greenhouse builders. Various greenhouse builders can also furnish this type of ground bed by contract.

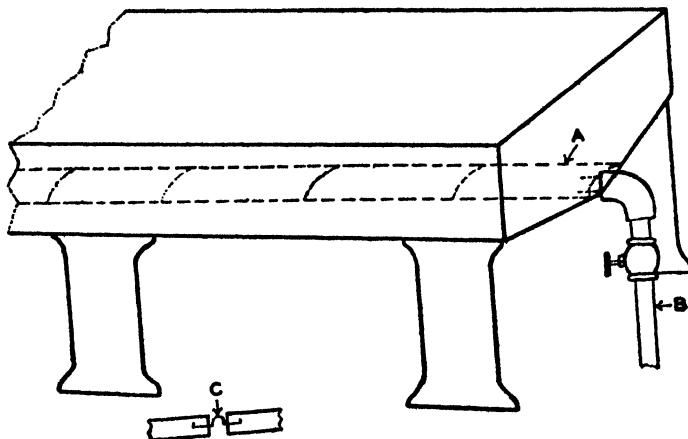


FIG. 4. V-BOTTOM TYPE, REINFORCED-CONCRETE BENCH WELL ADAPTED TO LARGE-SCALE GRAVEL CULTURE. IT MAY ALSO BE USED FOR SOLUTION OR SAND CULTURE

*A*—half-round, land tile; *B*—black-iron pipe line for delivery and return of nutrient solution; and *C*—top view of U-shaped copper or zinc expansion joint used to connect sections of the monolithic bench.

Withrow, Biebel, and Eastwood (25) have described the construction of asphalt beds which they claim can be formed directly in the ground at considerably lower cost than wooden or concrete ones. Such beds will be found relatively more economical for commercial use than for research purposes.

The fact that much of the piping to ground beds may be under ground, and some under the beds themselves, is a disadvantage to be considered when contemplating this type of equipment for research purposes. A type of bottom provided with a uniform slope to one side of the bed would virtually eliminate this objection.

In all benches and beds used for gravel culture, a uniform but slight slope lengthwise is desirable in that it facilitates the drainage of nutrient solutions from the bottom of the media.

The inner surfaces of all wood, metal, or concrete beds should be coated with asphalt or some similarly durable and inert material. Where cracks develop, a

mixture of hot asphalt and thoroughly dry sand makes a durable seal. Dry sand pressed into a fairly thick coat of asphalt paint, then covered by more paint, also gives good results.

Half-round land tile, roofing gutter, or similarly shaped material should be placed over the V-troughs in the beds, as described for sand cultures, to form channels through which the nutrient solutions are distributed freely.

*Nutrient solution tanks.* Cisterns or tanks for the preparation and storage of nutrient solutions are essential units in gravel culture. Their design and construction are precisely the same as those used in the subirrigation type of sand culture and discussed in that section of this paper. Again the importance of providing for a liberal volume of nutrient solution in relation to the area of gravel for which it is used warrants emphasis. Experience at the New Jersey Agricultural Experiment Station indicates that, in order to minimize the changes that occur in the solution after several applications to the gravel, and also to minimize the time required to reconstitute the solutions, at least 2 gallons of nutrient solution should be provided for each square foot of top surface in a bed.

*Pumping equipment.* Kiplinger and Laurie (15), Withrow, Biebel and Eastwood (25), and others have described in detail suitable pumps and various piping arrangements. Centrifugal pumps are employed for the most part. For research work, however, especially where flexible arrangement is desirable, the self-priming, sludge-type pumps should be considered. These pumps cost little more than centrifugal ones of similar capacity, are very durable and dependable, and have the advantage of not having to be placed low enough to enable the solutions to flow into them by gravity from the storage tanks. Sump-type pumps are also convenient to use, since they too require no separate motor and pump pits.

Where numerous small plots are required, the various solutions can be pumped simultaneously from a single motor connected by a V-belt to a drive shaft. Small, centrifugal pumps are available at moderate prices. These, in turn, can be driven by V-belts from the drive shaft.

Small plots can be operated conveniently by air pressure as illustrated in figure 2.

Although galvanized pipe has been used for the distribution of nutrient solutions in gravel culture for many years with no harm whatever to a variety of plants, there is still a possible risk associated with its use. Black-iron pipe is cheaper than the galvanized and is certainly nontoxic. Moreover, nutrient solutions appear to produce much less corrosion in black-iron pipe than do many drinking waters. For some types of investigations the use of aluminum pipe is advisable.

Bronze or brass fittings and valves may be objectionable in gravel cultures used for some investigations. For most purposes, however, their use appears to be entirely warranted. The likelihood to toxicity of plants resulting from their use with nutrient solutions containing moderate amounts of phosphorus appears to be very remote.

*Choice of gravel.* The purpose of the investigation may limit the choice of

gravel to be used. As is true with sand cultures, where maximum inertness is essential, virtually pure quartz gravel should be used. In general, with gravels composed of quartz, granite, or granite-like materials, an assortment of particle sizes ranging from  $\frac{1}{8}$  to  $\frac{1}{2}$  inch in diameter gives good results. Some traprocks are relatively inert and satisfactory for gravel culture. As measured by their greatest diameter, these materials should range in size from  $\frac{1}{8}$  to  $\frac{1}{2}$  inch. Haydite, a sintered shale, is relatively inert and is porous. An assortment of sizes of this material ranging from  $\frac{1}{8}$  to  $\frac{1}{2}$  inch in diameter makes an excellent root medium.

Well-fused soft-coal cinders are usually satisfactory. It is important, however, to examine them before they are used. Some contain toxic amounts of soluble boron. Most of them contain more or less sulfur which becomes oxidized to sulfuric acid. This may produce an undesirable change in the pH of the media unless it is neutralized promptly. When new cinders are used, this oxidation of sulfur often begins within the first 3 or 4 weeks and is usually completed in another 3 weeks. Some cinders weather rapidly and give rise to a large proportion of fine material. Because of these considerations, old, well-fused cinders that have been stored out-of-doors and exposed to weathering for several months or more are to be preferred. They should be washed and screened to an assortment of sizes ranging from  $\frac{1}{8}$  to  $\frac{1}{2}$  inch in diameter.

*Frequency of application of nutrient solution.* Environmental conditions and the species of plants grown play a very important part in determining the number of times the media should be flooded with nutrient solution daily. If the bed can be flushed and drained quickly, there is none but a practical limit to the number of times the solution may be applied daily. One must realize that the oxygen requirements of the roots of various plants differ greatly. The equipment should be designed to allow the benches to be flooded and nearly drained within half an hour. Slow flooding and draining may be particularly objectionable during hot weather.

In general, four applications of nutrient solution daily are adequate to support good growth in most crops grown in greenhouses. In some cases it may be desirable to apply the solutions less often as a means of controlling the quality of plant growth.

There is no advantage in flooding the top inch of media with nutrient solution. In fact, this should be avoided whenever possible, except where seedlings have been transplanted recently. Where the top of the gravel is kept moist with nutrient solutions, an objectionable growth of green algae will occur.

#### NUTRIENT SOLUTIONS

The concentration and balance of nutrients in the solution surrounding plant roots in artificial cultures vary more or less, depending upon the rate of application of the solution, or, in the case of solution cultures, upon the amount of circulation. This is particularly true of the nutrient substrate surrounding the roots of plants in gravel cultures. It is apparent, therefore, that the choice of one nutrient solution over another one varying from it slightly with respect to the concentrations of various major nutrients present is unwarranted. This statement is not in-

tended to minimize the importance of the balance of ions in a nutrient solution. The point to be emphasized, however, is that such a balance is concerned with a suitable range of concentrations of various major nutrients rather than with the strict maintenance of given concentrations of each.

This contention does not apply to the minor elements, or micronutrients, since small differences in the levels at which they are provided in nutrient solutions are known to effect pronounced changes in plant growth.

Probably the triangle system, employing the ammonium sulfate series of solutions of Jones and Shive (14), offers the most efficient method for selecting a good nutrient solution for the growth of species for which suitable ones have not previously been ascertained. In some instances it has been found advisable to employ lower concentrations of magnesium sulfate and of ammonium sulfate than were used by Jones and Shive. Such trials to determine a suitable nutrient solution can be made conveniently with small sand cultures.

The following nutrient solution was developed at the New Jersey Agricultural Experiment Station for the growth of roses in gravel culture. It represents an outgrowth of a somewhat similar initial formula modified several times on the basis of the results of continuous nutrient absorption and rose production studies extending over a period of 6 years. This solution has the following composition:  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 0.0005M;  $\text{Ca}(\text{NO}_3)_2$ , 0.0043M;  $\text{KNO}_3$ , 0.0077M;  $\text{MgSO}_4$ , 0.0035M; and  $(\text{NH}_4)_2\text{SO}_4$ , 0.0008M.

It is interesting to note the similarity of the above solution to one developed in a similar manner by Arnon and Hoagland (5) for the growth of tomatoes in solution culture. Their solution has the following composition:  $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.002M;  $\text{Ca}(\text{NO}_3)_2$ , 0.003M;  $\text{KNO}_3$ , 0.010M; and  $\text{MgSO}_4$ , 0.002M.

For several crops grown by gravel culture in setups possessing iron pipes and brass valves, initial quantities of Fe, Mn, and B equivalent to 5 gm.  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 1 gm.  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ; and 1 gm.  $\text{H}_3\text{BO}_3$  per 100 gallons of nutrient solution have produced excellent results. When a single tank of solution is to be used over a period of time, the micronutrient concentration is satisfied by the addition of 3 gm.  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  semiweekly; 1 gm.  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  weekly; and 1 gm.  $\text{H}_3\text{BO}_3$  every 12 weeks for each 100 gallons of solution.

When high-grade distilled water and especially purified C. P. salts were employed, and the solutions were protected from contact with metal equipment, Arnon (3) found it necessary to include 0.5 p.p.m. B, 0.5 p.p.m. Mn, 0.05 p.p.m Zn, and 0.02 p.p.m. Cu. Further purification of salts and water established the necessity of including molybdenum in solution cultures (4).

In general, the same nutrient solution found suitable for the growth of a group of plants in small-scale sand cultures will usually be found satisfactory for their growth in large solution, sand, or gravel cultures.

#### PLANTING

Robbins (19) has described methods for growing plants for research in sand culture. The same methods apply equally well for growing plants for use in large-scale artificial cultures.

In transplanting into gravel particularly, it is advisable to use small plants or dormant ones. They should be set with their roots well spread, but extended as deeply as possible.

No special precautions need be observed when setting plants in large sand cultures as compared with small ones. Planting into solution cultures was discussed previously in this paper.

#### METHODS USED FOR THE ANALYSIS OF NUTRIENT SOLUTIONS

*Nitrate-nitrogen:* Where no organic matter is present, nitrate-nitrogen may be reduced to ammonium-nitrogen by Devardas' alloy in aliquots diluted and made 0.25 *N* with NaOH. The ammonium-nitrogen present is distilled and estimated as in Kjeldahl determinations. Ammonium-nitrogen originally present in the aliquot must be deducted, as well as the blank value.

Where organic matter is present, nitrates are determined by the phenoldisulfonic acid method.

*Ammonium-nitrogen:* Ammonium-nitrogen is conveniently estimated by Nesslerization, using Jackson's modification (22, p. 653).

*Phosphate-phosphorus:* This may be estimated accurately and rapidly by the colorimetric method of Fiske and Subbarow (10). When a photoelectric colorimeter is employed, a deep red filter having a transmission range of 635 to 720 m $\mu$  is used.

*Potassium:* When determinations are made to obtain information on the amounts of potassium to add for nutrient maintenance purposes, the rapid turbidimetric method of Garman (11) gives a satisfactory degree of accuracy.

Where conditions warrant the use of a more accurate method than this, a combination of the procedures of Wilcox (24) and Sideris (21) is used. The color developed can be estimated very accurately in a photoelectric colorimeter equipped with a blue-green filter having a transmission value of 465 to 530 m $\mu$ .

*Calcium:* It has been found convenient to precipitate with saturated ammonium oxalate the calcium present in 5-ml. aliquots. Centrifuge and wash the precipitate repeatedly, and then estimate the oxalate present by titration with 0.01 *N* KMnO<sub>4</sub>.

*Magnesium:* The supernatant liquid from the first centrifugation of the calcium oxalate precipitate is used for estimating magnesium by the method of Hirschfelder and Serles (13). A photoelectric colorimeter is used, and readings are made through a yellow-green filter having a transmission range of 515 to 585 m $\mu$ .

#### INSECT AND DISEASE CONTROL

Large soilless cultures of all types can be sterilized conveniently and satisfactorily with a 1:100 solution of formaldehyde in water. It may be important not to attempt to sterilize beds or tanks by this method in the immediate vicinity of growing plants.

When sprays or dusts are necessary for insect or disease control, the user should know beforehand that they will not interfere with the investigation. There is a wide variety of effective materials from which a safe spray or dust can be chosen.

The practice of filling the tanks or beds with water and allowing them to overflow while applying sprays has not been found necessary and may lead to root injury during hot weather.

The control of pests by the use of safe fumigants is distinctly preferable to the use of sprays and dusts.

#### DISCUSSION

The use of large artificial cultures in plant research offers a degree of control over inorganic nutrition not attainable with soil. It also permits economy of time in growing large numbers of plants as well as large quantities of plant material. The choice of one method of artificial culture over another should depend upon the requirements of the individual investigation. Thus, solution cultures offer the highest degree of control over the composition of the nutrient substrate. They also permit convenient access to roots, as for sampling and making observations. Sand cultures permit greater ease of growing and supporting plants than do solution cultures, but at some sacrifice in refinement of nutrient control. Gravel culture offers about the same degree of control over the composition of the substrate as does the sand method. Depending upon the frequency of the pumping or flooding cycle, the changes occurring in nutrient concentration about the plant roots may be even less than in sand. Gravel cultures furnish the most favorable conditions of root aeration with a minimum of attention.

#### REFERENCES

- (1) ALEXANDER, L. J., MORRIS, V. H., AND YOUNG, H. C. 1939 Growing Plants in nutrient solution. *Ohio Agr. Exp. Sta. Spec. Cir.* 56.
- (2) ALLISON, R. V. 1922 The relation of aeration to the development of the soybean plant in artificial culture. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1921: 338-343.
- (3) ARNON, D. I. 1938 Microelements in culture-solution experiments with higher plants. *Amer. Jour. Bot.* 25: 322-325.
- (4) ARNON, D. I., AND STOUT, P. R. 1939 Molybdenum as an essential element for higher plants. *Plant Physiol.* 14: 599-602.
- (5) ARNON, D. I., AND HOAGLAND, D. R. 1940 Crop production in artificial culture solution and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. *Soil Sci.* 50: 463-485.
- (6) BIEKART, H. M., AND CONNORS, C. H. 1935. The greenhouse culture of carnations in sand. *N. J. Agr. Exp. Sta. Bul.* 588.
- (7) CHAPMAN, H. D., AND LIEBIG, G. F., JR. 1938 Adaptation and use of automatically operated sand-culture equipment. *Jour. Agr. Res.* 56: 73-80.
- (8) EATON, F. M. 1936 Automatically operated sand-culture equipment. *Jour. Agr. Res.* 53: 433-444.
- (9) EATON, F. M. 1941 Plant culture equipment. *Plant Physiol.* 16: 385-392.
- (10) FISKE, C. H., AND SUBBAROW, Y. 1925 The colorimetric determination of phosphorus. *Jour. Biol. Chem.* 66: 375-400.
- (11) GARMAN, W. H. 1943 Photronic method for determining potassium in soils and plants. *Soil Sci.* 56: 101-107.
- (12) GERICK, W. F. 1940 A Complete Guide to Soilless Gardening. Prentice-Hall, Inc., New York.
- (13) HIRSCHFELDER, A. D., AND SEELES, E. B. 1934 A simple adaption of Kolthoff's colorimetric method for the determination of magnesium in biological fluids. *Jour. Biol. Chem.* 104: 635-645.

- (14) JONES, L. H., AND SHIVE, J. W. 1921 Effect of ammonium sulfate upon plants supplied with ferric phosphate and ferrous sulphate as sources of iron. *Jour. Agr. Res.* 21: 701-728.
- (15) KIPLINGER, D. C., AND LAURIE, A. 1942 Growing ornamental greenhouse crops in gravel culture. *Ohio Agr. Exp. Sta. Bul.* 634.
- (16) LEBEDEFF, A. F. 1927 The movement of ground and soil waters. *Proc. 1st. Internat. Cong. Soil Sci.* 1: 459-494.
- (17) NIGHTINGALE, G. T. 1934 Ammonium and nitrate nutrition of dormant Delicious apple trees at 48°F. *Bot. Gaz.* 95: 437-452.
- (18) ROBBINS, W. R. 1937 Relation of nutrient salt concentration to growth of the tomato and to the incidence of blossom end rot of the fruit. *Plant Physiol.* 12: 21-50.
- (19) ROBBINS, W. R. 1946 Growing plants in sand cultures for experimental work. *Soil Sci.* 62:
- (20) SHIVE, J. W. 1941 The balance of ions and oxygen tension in nutrient substrates for plants. *Soil Sci.* 51: 445-459.
- (21) SIDDEBS, C. P. 1937 Colorimetric microdetermination of cobalt and potassium. *Indus. and Engin. Chem., Analyt. Ed.* 9: 145-147.
- (22) SNELL, F. D., AND SNELL, C. T. 1936 Colorimetric Methods of Analysis. D. Van Nostrand Co., New York.
- (23) THOMAS, M. D. 1943 An installation of large sand-culture beds surmounted by individual air-conditioned greenhouses. *Plant Physiol.* 18: 334-344.
- (24) WILCOX, L. V. 1937 Determination of potassium by means of an aqueous solution of trisodium cobaltinitrite in the presence of nitric acid. *Indus. and Engin. Chem., Analyt. Ed.* 9: 136-138.

# DESIGNS OF GREENHOUSE EXPERIMENTS FOR STATISTICAL ANALYSIS<sup>1</sup>

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Numerous greenhouse studies of soil deficiencies and plant needs are being conducted by experimenters in agronomy, plant pathology and physiology, soil science, and horticulture. Although greenhouse experiments may be performed for observational or exploratory purposes, most experiments brought to the attention of the statistician are investigations to determine small differences. Such experiments call for improved techniques, efficient methods of layout, and careful analysis of results.

In this paper some types of layout that have been applied successfully in greenhouse experiments are illustrated. The object in selecting an arrangement on the greenhouse bench, like the object of a good technique, is to obtain the maximum accuracy commensurate with the amount of time and labor available for the conduct of the experiment. In both instances, the first step toward increased accuracy is to discover the principal causes of the variation observed in the greenhouse.

## SOURCES OF VARIATION IN GREENHOUSE EXPERIMENTS

Variation within a greenhouse bench may be large, though it has been considerably reduced in the newer greenhouses built specifically for comparative tests. The major sources of variation are temperature gradients caused by such things as steam pipes under the bench, doors, and proximity to ventilators; shading effects resulting from the structures in the house, adjacent buildings or trees; and moisture differentials caused by methods of watering and air currents.

A careful appraisal of the greenhouse facilities and some experience in experimentation will usually indicate rather accurately which of these sources of variation exist. The nature and direction of environmental gradients will also be apparent. For example, steam pipes under each edge of a bench and running parallel to the bench will produce sharp gradients within 3 to 5 feet across the bench. LeClerg (6) has given a striking example of differences in the stands of sugar-beet seedlings caused by a temperature gradient from vertical radiating coils on the side walls and ends of the greenhouse. On a bench adjacent to an outside ventilator a marked temperature and possibly a moisture gradient will exist across the bench. Of course, such variations are most pronounced in greenhouses located where extreme climatic conditions prevail.

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Shading effects are rather universal and usually are greater along the bench than across it. Sometimes they can be avoided by not using that part of the bench near structural material. Another important source of variation arises from inequalities in watering (methods of watering are discussed elsewhere in this issue).

In addition to the effects of temperature, shading, and moisture inequalities, there remains a serious source of error which is summed up in the phrase "pot to pot variation." Often this variation is due mainly to inherent differences in the responsiveness of individual plants; because of the small numbers of plants per pot which are typical in greenhouse experiments, these plant differences are not averaged out to anything approaching the extent that occurs in a plot in a field experiment. Here again, however, much may be accomplished by good technique; for instance, by weighing the soil into the pots for fertilizer experiments and, when the plants are large, by leaving sufficient space between pots to mitigate the effects of interpot competition (or contamination in disease experiments). More experimental work on the selection of a suitable pot size might be profitable, though experience has given useful leads.

With a knowledge of the greenhouse conditions that will affect the experimental results and an appreciation of the limitations of the experimental unit to be used, the research worker is in a position to select an efficient experimental design.

#### MODERN EXPERIMENTAL DESIGNS

From the point of view of their application to greenhouse experiments, modern experimental designs may be divided into two classes. In the first class, which contains randomized blocks, lattice designs, and balanced incomplete blocks, the experimental units (the pots, jars, or sections) are arranged in a series of groups in such a way that differences among the groups, whether environmental or associated with the plants, can be eliminated from the experimental errors. Such designs may be used to counteract the effects of a single gradient either along or across the bench or of differences between benches when the experiment extends over several benches. Where the treatments are applied to mature or partly mature plants, plant differences can also be eliminated to a considerable extent by placing similar plants within the same group. Naturally, the groups should be constructed so that the differences among groups represent what is considered to be the greatest source of variation. In the second class, consisting of latin squares, lattice squares, and Youden squares, the experimental units are arranged in groups in two different ways. Variations associated with both types of grouping are removed simultaneously from the errors, so that these designs can cope with gradients both along and across the bench. A brief description follows of some useful designs in each class, accompanied by an example of a greenhouse experiment.

The randomized block design is the simplest and most widely used design; indeed, there would be little need to consider other designs except that in certain circumstances they give a marked increase in accuracy over randomized blocks. In this design the experimental units are arranged in groups, each of which constitutes a complete replication of the treatments. Any number of treatments

and any number of replications may be used. An example may be selected from a group of floriculture experiments. A project was planned to determine whether differences existed among seven soil types in their suitability for use in commercial greenhouses. The soil was mechanically mixed and placed on the benches, which had been divided into sections of equal size. There were three replications, each containing seven sections. The soil types were assigned to the units, a new randomization being used for each replicate. For snapdragons 18 plants, for chrysanthemums 9 plants, and for stock 36 plants were placed in each section. Some of these experiments were conducted during the winter in a greenhouse located where the outside temperature was frequently below freezing. The heating pipes were at one end of the bench, and the other end was close to outside windows. The experiments gave a pictorial demonstration of bench gradients showing open flowers, half-open flowers, and buds from the warmer to the cooler end of the bench. The arrangement of the replicates removed most of this bench variation from experimental error.

In the *latin square* design the treatments are grouped into replications in two different ways. This design has been used for virus experiments where individual leaves are inoculated. In such experiments the position of the plant on the bench has little influence on the responses of its leaves to the inoculation; the important factors are plant differences and differences in the responses of leaves of different sizes. By the *latin square* arrangement the treatments can be placed so that each appears on every plant and on every leaf size. For plants with five usable leaves, a layout for five treatments (A, B, C, D, E) is shown below. The symbol (a) denotes the largest leaf on a plant, (b) the second largest, and so on.

SIZE OF LEAF	PLANT				
	1	2	3	4	5
(a)	A	C	B	E	D
(b)	E	D	C	A	B
(c)	D	A	E	B	C
(d)	C	B	A	D	E
(e)	B	E	D	C	A

For bench experiments the utility of *latin squares* with more than five treatments is limited by the narrowness of the bench. The larger *latin squares* may be useful in greenhouses that contain no bench but where large earthenware jars are used. For example, seven seed treatments were applied to a variety of soybeans. Lots of 50 seeds, one lot treated in each of the seven ways, were planted in gallon jars which were arranged in seven rows of seven jars each. Every treatment appeared in each row and in each column. The experiment covered considerable space, and the arrangement provided an opportunity to measure the environmental variations along and across that section of the greenhouse. Heat and light gradients were present. The difference produced by these gradients would have masked the treatment effects in a less appropriate design.

If the conditions of the experiment make it necessary to apply some treatments

to a considerable area on the bench, while others can be applied to a single pot, the two sets of treatments can be combined in the same trial by a device known as *split-plot* Technique. An experiment was carried out to test six new selections of peas grown with three different amounts of light—light for 24 hours, light for 12 hours, and total darkness. The lighting equipment introduced the limitations which made a split-plot design suitable. Two benches (replications) were partitioned into three sections each. At random, one section on each bench was surrounded by curtains to provide a dark condition. The other two sections were provided with lighting equipment to control the length of light for 12 or 24 hours. Within each section six pots, each containing seed from one of the selections, were placed at random.

For comparisons of the effects of different amounts of light, the experimental unit (sometimes called the *whole-unit*) is a section of the bench; for comparisons among the selections the experimental unit (sometimes called the *sub-unit*) is a single pot. In the analysis of variance, separate estimates of error are calculated for the two types of unit. The whole-unit error applies to the light treatments, the sub-unit error to the selections and to the interactions between the selections and the light treatments. Except in rare cases the sub-unit error is the smaller.

Another application of this technique occurs in studies of nutrient solutions using sand cultures, where it is impractical to attach the electric pumps to small experimental units. Other treatment comparisons may be introduced within each solution. In fact, when practicable an additional set of treatments may be added to any experiment by subdividing the whole-unit. A simple example of the statistical analysis is given by Hayes and Immer (5), a more complex one by Goulden (4).

#### INCOMPLETE BLOCK DESIGNS

Suppose that the width of the bench accommodates four pots and that a steep gradient has been found to exist along the bench. This gradient can be removed rather successfully by either randomized blocks or a latin square, provided that the number of treatments does not exceed four. With 16 treatments, however, there might be a substantial gradient within replicates in a randomized block layout. A similar difficulty arises when the treatments are applied to a heterogeneous batch of plants or shoots where it might be possible to assemble small groups of similar plants or shoots but not large groups. For instance, in experiments described by Morrow (8) on the comparison of self- and cross-pollination in blueberries, paired shoots of equal size and with approximately the same number of fruit buds were selected from the same plant in order to eliminate plant variation and variation in shoot vigor. If it were necessary to assemble groups of 20 "similar" shoots for the comparison of 20 treatments in randomized blocks, uniform matching within a group would have to be sacrificed to a considerable degree.

In this situation it is desirable to use an arrangement that makes possible the

elimination from the error of differences among groups that are smaller than a replication. A series of designs of this type have been developed by Yates (12) since 1936. Since the small groups or blocks do not contain all the treatments, the designs are sometimes called *incomplete block designs*.

One such design, a *balanced lattice*, was used for an experiment on the effects of eight hormone treatments on the development of tobacco plants grown in each of two soil types. Small (6½-inch pots) were used, and the plants were thinned to six per pot. Since environmental variations across the bench were expected, the 16 treatment combinations—1, 2, . . . , 16—were placed on the bench in the following order:

<i>Rep. I</i>	<i>Rep. II</i>	<i>Rep. III</i>	<i>Rep. IV</i>	<i>Rep. V</i>
9 1 5 13	13 12 6 3	5 6 7 8	3 10 16 5	13 7 10 4
16 8 12 4	1 10 15 8	14 16 13 15	9 4 15 6	11 1 16 6
15 7 11 3	14 4 11 5	11 12 9 10	13 8 2 11	15 12 2 5
14 6 2 10	16 2 7 9	2 1 4 3	12 1 14 7	8 9 14 3

The incomplete blocks, containing four pots each, were arranged to remove differences across the bench, while the replications removed a considerable amount of the variation along the bench. The results indicated less bench effect on the roots than on the tops, perhaps because light and temperature differences affect the tops in a more direct manner; also the roots were limited in space and the tops were not.

This design belongs to the group of *lattice designs*, which have some useful properties. These designs require no more work in the greenhouse than randomized blocks with the same number of replicates. The results can be analyzed, if desired, by the usual procedure for randomized blocks, though in general more accurate estimates of the treatment effects are obtained by use of the analysis appropriate to lattice designs. The essential feature of this analysis is that the treatment means are adjusted for environmental differences among the incomplete blocks. The method of analysis is given for the balanced lattice by Wellhausen (9) and for other lattices by Cox, Eckhardt, and Cochran (1). The chief limitation to the utility of the design is that the number of treatments must be an exact square.

*Balanced incomplete block designs* are, in general, similar to balanced lattices but are not restricted to numbers of treatments which are exact squares. An example on soybeans is shown in the following diagram; glazed pots 12 inches in diameter were used with 10 plants per pot:

*Bench I*

Lime 1		4	2	
b	c	Mg <sub>1</sub>	Mg <sub>2</sub>	Mg <sub>3</sub>
d	a			
c	a	Mg <sub>3</sub>	Mg <sub>1</sub>	Mg <sub>2</sub>
b	d			

*Bench II*

3	1	2

*Bench III*

3	4	2

*Bench IV*

1	3	4

Four soil types (a, b, c, d), two rates of Mg, and four levels of lime were tested in all combinations, giving 32 treatments. Since this experiment was intended principally to give information on the soil types and on differences in the responsiveness of the soil types to Mg and to lime, a double split-plot arrangement was used. The soil types were tested in individual pots, the Mg rates in groups of four neighboring pots, and the lime rates in groups of eight pots.

The space available did not allow 32 pots to be placed in the same bench so as to leave sufficient space between pots. Consequently, in a randomized blocks layout of the lime rates, each replication would have extended over more than one bench. For this reason the lime rates were arranged in the balanced incomplete block design for four treatments in blocks of three units. Each block of 24 pots could be placed on a single bench. The arrangement provided three replications and occupied four benches. Differences among benches were removed from the experimental error by means of adjustments applied in the statistical analysis to the results for the lime rates. An index to balanced incomplete block designs and an account of the method of analysis are given by Fisher and Yates (3).

By a rearrangement of certain of the balanced incomplete block plans, incomplete block layouts are obtained which remove differences between two types of grouping of the pots. This rearrangement (usually called a *Youden square*) is illustrated below for the lime rates design in the previous example:

Balanced Incomplete Blocks Blocks				Youden Square Blocks				
I	II	III	IV	Replicates	I	II	III	IV
1	3	3	1	I	1	2	3	4
4	1	4	3	II	4	3	2	1
2	2	2	4	III	2	1	4	3

Without any change in the compositions of the four blocks, the rows now represent separate replicates, so that 2 degrees of freedom may be subtracted from the error for differences among replicates in addition to the 3 degrees of freedom for blocks. A single replication of this design is not recommended, since only 3 degrees of freedom remain for error. By a duplication of the design, however, six replicates provide nine error degrees of freedom, while nine replicates provide 15 error degrees of freedom. The basic plans for these designs and some interesting applications to greenhouse experiments are described by Youden (13).

Similarly, *lattice squares* (11) are constructed by a rearrangement of the balanced lattices. In addition to the removal of differences among replicates, these designs provide adjustments for differences among rows and columns within replicates, thus allowing a high degree of control over the major sources of variation.

#### FACTORIAL EXPERIMENTS

Fisher (2) and Yates (10) have drawn attention to the advantages to be gained by investigating the effects of a number of different factors in the same experiment. In a *factorial* experiment, all combinations of the different factors are tested: the two split-pot experiments described above are examples. Efficient use is made of the experimental material, because every pot contributes information on all the factors under investigation. For instance, it was desired to measure the top and root growth of four species of grass on soil of high and of low fertility with four intensities of cutting. The three factors (four species, two fertility levels, four intensities of cutting) give 32 treatment combinations which were laid out in randomized blocks with four replications. There was considerable pot-to-pot variation within replicates; the uneven stands indicated variation in moisture content, crusting, and disease at planting. Nevertheless, the average effects of the factors were estimated with a satisfactory degree of accuracy because the experiment afforded 32 replications for the species and intensities comparisons and 64 replications for the comparison of the fertility levels. Also, by a study of the first-order interactions, it was possible to test whether the species responded similarly to the different intensities of cutting and to the two fertility levels and whether the effect of intensity of cutting was conditioned by the fertility level.

Factorial experiments may be arranged in randomized blocks, in latin squares, or in split-plot designs. If the total number of treatment combinations is so large that some reduction in block size is advisable, the incomplete block designs described above are suitable when the study of interactions is considered as important as that of the average effects of the factors. For many crops, however, experimentation has indicated that the high-order interactions are usually negli-

gible or relatively small. In such cases it is preferable to reduce block size by a sacrifice of information on these interactions. Perhaps the most useful designs of this type are those for a confounded  $2 \times 2 \times 2$  factorial experiment in blocks of four pots. This design was used on corn to study the root-top ratio of an inbred line (a slow-growing genetic inbred) compared with a very vigorous, uniform hybrid. These two sources of seed were grown in poor and in good soil, kept in wet and in dry condition. This gave a  $2 \times 2 \times 2$  factorial consisting of two seed sources, two fertility levels, and two moisture conditions. It was known that considerable variation existed along the bench and since only four pots (6 inch) could be placed across it, the experiment was arranged in incomplete blocks of four pots across the bench. Two adjacent blocks made a replication, of which there were four. In this experiment there are three first-order interactions and one second-order interaction, each of which was confounded with blocks in one of the four replications. In effect, the experiment gives four replicates on the average effects and three replicates on the interactions. A more common variant is to confound the second-order interaction in all replicates, giving greater reliability to the measure of main effects and first-order interactions. Yates (10) gives a systematic account of confounded factorial designs, and Li (7) contributes some additional arrangements.

In the foregoing experiment, the pots within the incomplete blocks were re-randomized at intervals during the early part of the trial. It is now believed that this was unnecessary. The ability to move pots around is an advantage of greenhouse conditions over field conditions, and may be used in an attempt to equalize environmental conditions or to avoid the worst effects of inter-pot competition. Unfortunately, we have found no data on the effectiveness of the practice: its drawbacks are the labor involved, the possibility of injury to the plants, and the opportunity for unobserved biases. The use of incomplete block designs should render the practice of less value insofar as homogeneity within the blocks is attainable.

#### NUMBER OF REPLICATIONS

Greenhouse experiments are less accurate than is usually realized, particularly where crop weights are concerned. Not infrequently the percentage standard errors per pot are higher than those per plot in field experiments with the same crop. When the resources of skillful technique and layout have been exhausted, the only way in which accuracy can be increased is to provide more replications.

It is not easy to give helpful advice on the desirable amount of replication. Often a compromise must be made between the conflicting claims of different experiments; moreover, the issue is complicated when it is intended to repeat the experiment several times if initial results appear promising. In the simplest case, considering only a single experiment, we require for a realistic discussion an estimate of the standard error per pot, which can often be made from previous experience, and a statement of the size of difference which the experiment is designed to detect and measure.

Suppose that the standard error per pot is 10 per cent of the average yield—

a figure not unusually high for greenhouse experiments—and consider how accurately treatment differences are measured in an experiment with four replications. The standard error of a treatment mean is  $10/\sqrt{4}$  or 5 per cent and that of the difference between two treatment means is  $5\sqrt{2}$  or about 7 per cent of the average yield. The difference which will be observed in the experiment varies about the true difference with this standard error. If the number of error degrees of freedom is ample, we find from the normal probability tables that four times out of five the observed difference will vary from the true difference by less than  $7 \times 1.28 = 9$  per cent of the average yield. In other words, the experiment is fairly certain to determine the true difference to within  $\pm 9$  per cent of the average yield.

For instance, if the true difference is 10 per cent of the average yield, the difference observed in the experiment is fairly certain to lie somewhere between  $(10 - 9)$  and  $(10 + 9)$  per cent; that is, between 1 per cent and 19 per cent. It is clear that this experiment cannot be expected to measure a true 10 per cent difference with any degree of precision, since the experiment might show a difference as low as 1 or 2 per cent, or a difference as large as 18 or 19 per cent. Similarly, if the true difference happens to be 30 per cent, the limits for the observed difference are  $(30 \pm 9)$  per cent; that is, 21 per cent and 39 per cent. It follows that the experiment can distinguish, with reasonable certainty, between a true difference of 10 per cent and one of 30 per cent, because in the former case the observed difference is unlikely to exceed 19 per cent, whereas in the latter case the observed difference is unlikely to be lower than 21 per cent. The experiment cannot be relied upon, however, to discriminate between true differences of 10 and 20 per cent.

This kind of calculation gives some idea of the accuracy with which experiments having a given amount of replication will measure real treatment differences of specified sizes. Alternatively, in setting up a preliminary experiment, we may wish to know the number of replications necessary in order that a true difference of given size is fairly certain (say four times out of five) to be detected as significant at the 5 per cent level. In the foregoing example, with four replicates and a 10 per cent standard error per pot, it is found that a true difference of 21 per cent satisfies these conditions; that is, four times out of five the observed difference will exceed the value required for 5 per cent significance. With a true difference of 10 per cent, on the other hand, calculation shows that there is considerably less than a 50-50 chance that the experiment will indicate a significant difference. Thus it is quite likely that the existence of a true 10 per cent difference would be undiscovered in a preliminary experiment of this type.

To visualize what is involved in this sort of problem it is necessary first of all to compute the smallest difference that would be judged significant at the 5 per cent level by the *t* test. In the present example the standard error of the difference between two treatment means is 7.1 per cent of the average yield. Assuming that there are eight treatments in randomized blocks, with four replicates per treatment, the number of degrees of freedom for estimating error is  $7 \times 3 = 21$ . For 21 degrees of freedom the value of *t* required for significance is 2.080. The

smallest difference that would be considered significant is, therefore,  $2.08 \times 7.1 = 14.8$  per cent. To achieve a significant difference at least four times out of five trials, it is then necessary to get an observed difference as large as 14.8 per cent at least four times out of five trials. If the standard error of an observed difference is 7.1 per cent, and an observed difference of 14.8 per cent or more is to be attained four times in five trials, the true difference in the population must satisfy the relation  $\frac{14.8 - d}{7.1} = -0.859$ . The number  $-0.859$  represents the value of  $t$  which will be equalled or exceeded four times in five trials when the true difference in the population is  $d$  and the standard error of the observed difference is estimated from 21 degrees of freedom. Solving this equation gives  $d = 20.9$  per cent. Thus the true difference in the population must be at least 20.9 per cent before an observed difference of 14.8 per cent will be reached as often as four times in five trials, or, in other words, the true difference in the population must

TABLE 1

*Number of replications required in order to obtain a significant observed difference (5 per cent level) in four experiments out of five*

TRUE DIFFERENCE (AS % OF AVERAGE YIELD)	STANDARD ERROR PER CENT PER UNIT (POT, SECTION, ETC.)					
	14	12	10	8	6	4
5	124	91	63	41	23	11
10	31	23	16	11	6	3
15	14	11	8	5	3	2
20	8	6	5	3	2	2
30	4	3	3	2	2	2

be at least 20.9 per cent if a significant difference (5 per cent level) is to be achieved at least four times in five trials.<sup>3</sup>

Table 1 shows the number of replications required in order to be fairly certain (four times out of five) of obtaining a significant observed difference when the true difference is 5, 10, 15, 20, and 30 per cent, respectively. The table has been prepared for a series of standard errors per unit (pot, section, etc.) which cover the range usually encountered in greenhouse experiments.<sup>4</sup>

With three or four replications, it is evident that we can expect to detect only true differences of 20 per cent or more, unless the standard error per unit is un-

<sup>3</sup> This argument is not exactly correct. In an actual experiment, where the true standard deviation is unknown, the difference required for significance is not 14.8 per cent, but 2.08 times the estimated standard error of the difference between two treatment means. The argument, however, displays the nature of the problem and gives a very close approximation to the exact result, which requires more complicated mathematics. Values in table 1 were based on the correct argument, for which see Neyman, *Sup. Jour. Roy. Statist. Soc.*, 2, 1935.

<sup>4</sup> In taking into account the number of error degrees of freedom, the experiment was assumed to have eight treatments in randomized blocks; thus with  $r$  replicates, there are  $7 \cdot (r - 1)$  error degrees of freedom.

usually small. To cope with true differences of 5 or even 10 per cent, large numbers of replications are required throughout most of the range of standard errors per unit. Moreover, it should be remembered that these numbers of replications merely insure, with reasonable certainty, that the experiment will indicate the existence of a true difference. If, in addition, the experiment is to estimate the size of the true difference within narrow limits, the requirements in number of replications are considerably higher.

The table serves to emphasize that fine differences cannot be determined with few replications. The value of any reduction in standard error obtainable by good technique or layout is also apparent, as is the efficiency of factorial experiments, where there may be from 20 to 60 replications for the average effects of a factor.

#### SUMMARY

For accurate experimentation under greenhouse conditions, a knowledge of the environmental variations due to the structure and location of the greenhouse and of the variability of the plant material is of prime importance. Brief descriptions are given, with illustrations from greenhouse experiments, of a number of experimental designs that enable the experimenter to utilize this knowledge so as to eliminate the major sources of variation from the experimental errors. An investigation is made of the accuracy of experiments with a given number of replicates. This study shows that under typical greenhouse conditions only large treatment differences can be detected with three or four replicates and makes evident the value of any reduction in standard error per pot obtainable from improved techniques or designs.

#### REFERENCES

- (1) COX, G. M., ECKHARDT, R. C. AND COCHRAN, W. G. 1940 The analysis of lattice and triple lattice experiments in corn varietal tests. *Iowa Agr. Exp. Sta. Res. Bul.* 281.
- (2) FISHER, R. A. 1942 *The Design of Experiments*, ed. 3. Oliver and Boyd, Edinburgh.
- (3) FISHER, R. A., AND YATES, F. 1943 *Statistical Tables*, ed. 2. Oliver and Boyd, Edinburgh.
- (4) GOULDEN, C. H. 1939 *Methods of Statistical Analysis*. John Wiley and Sons, New York.
- (5) HAYES, H. K., AND IMMER, F. R. 1942 *Methods of Plant Breeding*. McGraw-Hill, New York.
- (6) LE CLERC, E. L. 1935 Factors affecting experimental error in greenhouse pot tests with sugar beets. *Phytopath.* 25: 1019-1025.
- (7) LI, J. C. R. 1944 Design and statistical analysis of some confounded factorial experiments. *Iowa Agr. Exp. Sta. Res. Bul.* 333.
- (8) MORROW, E. B. 1943 Some effects of cross-pollination versus self-pollination in the cultivated blueberry. *Proc. Amer. Soc. Hort. Sci.* 42: 469-472.
- (9) WELLHAUSEN, E. J. 1943 The accuracy of incomplete block designs in varietal trials in West Virginia. *Jour. Amer. Soc. Agron.* 35: 66-76.

- (10) YATES, F. 1937 The design and analysis of factorial experiments. Imp. Bur. Soil. Sci. Tech. Commun. 35.
- (11) YATES, F. 1940 Lattice squares. *Jour. Agr. Sci.* 30: 672-687.
- (12) YATES, F. 1940 Modern experimental design and its function in plant selection. *Empire Jour. Exp. Agr.* 8: 223-230.
- (13) YOUDEN, W. J. 1940 Experimental designs to increase accuracy of greenhouse studies. *Boyce Thompson Inst. Contrib.* 11: 219-228.

# PHOTOPERIODIC RESPONSE AS A FACTOR IN CHOICE OF PLANTS FOR TESTING SOIL DEFICIENCIES

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Many of the plants used in greenhouse experiments dealing with soil deficiencies, comparisons of nutrient solutions, and similar studies develop differently depending upon the season during which they are grown. These differences are expressed both in amount of growth and kind of development and are the result of seasonal variations in a number of environmental factors, chief among which are temperature and light. Insofar as light is concerned, differences in amount of growth are to be attributed chiefly to differences in intensity or total energy of light received by the plant. Differences in kind of development are mainly the result of differences in the daily duration of light, or photoperiod.

The differences in plant development resulting from seasonal variations in photoperiod are often so great that it becomes impractical to conduct studies with some kinds of test plants at certain times of the year. Thus the natural photoperiod prevailing in winter may prevent flowering and fruiting of certain plants during that season. With other plants it may cause the habit of growth to change from erect to prostrate, and with still others it may prevent formation of bulbs or other storage organs.

Although photoperiod may result in very marked differences in the development of many plants, methods are available by means of which this source of seasonal variability can be largely avoided or prevented. The purpose of this paper is to discuss these methods and to describe the photoperiodic responses of a number of kinds of plants that have been used or perhaps could be used advantageously for testing soil deficiencies.

## DEFINITIONS

A number of terms frequently encountered in literature dealing with photoperiodism may well be considered at this point in order that their meanings as used in this paper may be clear.

The successive periods of light and darkness to which plants are exposed are referred to respectively as *photoperiods* and *dark periods*. Since very brief durations of light have been shown to cause rather remarkable effects on plant development, it is convenient to regard any uninterrupted light period as a *photoperiod*, regardless of the duration or source of the light. Thus photoperiods may vary in length from less than a minute to many hours and they may occur at daily intervals or with greater or less frequency. They may be composed entirely of either natural or artificial light or may include parts of each. Such a definition of a photoperiod implies that the dark periods likewise must be

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defined as continuous. A dark period of 16 hours, interrupted in the middle by a minute of light would therefore be regarded as two dark periods of 8 hours separated by a 1-minute photoperiod.

Garner and Allard (4) classified plants according to their photoperiodic responses as long-day, short-day, and indeterminate types. By a *long-day plant* is meant one which flowers when subjected to photoperiods longer than a certain minimum, known as the *critical photoperiod*, and which fails to flower or is greatly delayed in flowering when subjected to photoperiods shorter than the critical. A *short-day plant* on the other hand, flowers on photoperiods shorter than the critical and fails to flower on longer photoperiods. *Indeterminate plants* are those whose flowering is not appreciably influenced by the length of photoperiod they receive.

#### CHOICE OF VARIETY, A MEANS OF AVOIDING SEASONAL VARIABILITY

The photoperiodic responses of numerous plants have been investigated since since Garner and Allard published their first paper on the subject in 1920. For various reasons, certain kinds of plants have been studied far more extensively than others in this respect, with the result that the photoperiodic responses of some are known in considerably more detail than those of others. These findings may or may not hold true in detail for most plants that are photoperiodically sensitive, but general principles can be set forth that will serve as a guide in the selection of plants which, insofar as their photoperiodic responses are concerned, should be suitable for testing soil deficiencies.

The most obvious method of avoiding variability in growth and development of test plants caused by seasonal changes in length of photoperiod is to select a plant that is photoperiodically indeterminate. If the object of the experiment allows a choice, consideration may profitably be given to selection of such a plant. The tomato, which has been used extensively in various types of nutrition studies in the greenhouse, is indeterminate, flowering and fruiting over a wide range of day-lengths. This characteristic of the tomato has probably been one of the important reasons for its extensive use in such studies. Many of the varieties of beans of the genus *Phaseolus*, reported as day-neutral by Allard and Zaumeyer in a recent extensive study (1), should also be worthy of consideration for use as test plants for this reason. If plants of the indeterminate type can be used in greenhouse studies, one of the principal causes of extreme seasonal variability can thus be avoided.

Experimental conditions, however, sometimes require the use of a kind of plant that is not indeterminate in its photoperiodic response. In such cases, if a number of varieties are equally acceptable insofar as other experimental considerations are concerned, it may be possible to select from them one that is considerably less influenced than the others by the particular range of natural photoperiods encountered in the latitude where the tests are to be made. This condition is well illustrated by the soybean.

In general, all varieties of soybeans are to be considered as short-day plants because their flowering is accelerated if they are subjected to short days and is

delayed or inhibited by long days. It is true that some soybean varieties are capable of flowering under continuous light and for this reason might be considered as indeterminate, but since these varieties flower much earlier if they are subjected to short photoperiods it seems more appropriate to consider them as short-day types. The variety Agate is one of those that flowers under continuous light. In the field this variety matures seed as far north as Winnipeg, Canada, and although it does not fruit abundantly in the greenhouse under continuous light it does so when subjected to controlled daily photoperiods of 18 hours. The variety Biloxi, on the other hand, does not mature seed in the field in the northern half of the United States because of its requirement of short photoperiods for flowering. When the natural photoperiods of that region are sufficiently short to permit flowering, there is insufficient time for the crop to mature before frost. In the greenhouse, plants of this variety flower if the photoperiods do not exceed about 14 hours daily, but they remain vegetative almost indefinitely when grown on longer photoperiods. For obtaining less variable development over a wide range of natural day-lengths, varieties such as Agate are therefore superior to those of the Biloxi type. This condition is not confined to soybean varieties alone, but occurs in numerous other kinds of plants, some of which are discussed later.

#### REGULATION OF GROWTH BY ARTIFICIAL CONTROL OF PHOTOPERIOD

If experimental requirements make it necessary to use a plant that responds strongly to the prevailing seasonal variations in day-length, then it is possible by means of inexpensive equipment and a comparatively simple routine so to regulate photoperiod that the plants either will continue a vegetative development or will flower and fruit, as may be desired. Thus the experimenter can eliminate much of the effect of seasonal variability encountered when such a crop is grown under conditions of natural illumination and at the same time he can maintain his plants in either a vegetative or a reproductive condition according to the requirements of his experiment. Vegetative development of a variety such as Biloxi soybean can, for example, be ensured during seasons of short days by extending the photoperiods with artificial light, and early flowering can be induced during the periods of long natural photoperiods by darkening the plants each day after they have received 8 or 10 hours of daylight. By such manipulation of the photoperiod, it thus becomes relatively easy to grow fairly uniform test plants throughout the year. Tests can, therefore, be made with plants of a single variety in either a vegetative or a reproductive stage of development or simultaneous tests can be made with plants in each condition. Since the nutrient requirements of vegetative plants are probably considerably different from those of plants in a reproductive condition this latter procedure would appear to have considerable merit.

If plant development is to be regulated by extending the natural photoperiod, then the duration and intensity of artificial light and the time at which it is applied become important considerations. When it is necessary to shorten the natural photoperiod, the minimum intensity of light to produce effects of

darkness must be accurately known. Both the duration and intensity requirements differ between long-day and short-day groups and among species in each group. With Biloxi soybean, flowering is prevented if short days are lengthened to approximately 14 hours by means of supplemental light. This results in a decrease in length of the dark period to about 10 hours daily. The intensity of the supplemental light need not exceed 40 foot-candles to be effective with the Biloxi soybean, and under ideal conditions it may safely be far less. It is usual practice to illuminate the plants from sundown until midnight, although light may be used equally well from midnight until morning. This duration of light is far in excess of that required to prevent flowering of Biloxi soybean if the light is applied at the proper time. A period of light as brief as 1 minute nightly is sufficient to prevent flowering if it occurs near the middle of the night and thus divides the night into two dark periods, each of which is less than about 10 hours in length, and if every leaf surface receives an intensity of 5 to 10 foot-candles during this minute of illumination. Similar results have been obtained with chrysanthemum, but the intensities and durations required appear to be somewhat greater.

Prevention of flowering of certain short-day plants is not so simple as with Biloxi soybean. Thus the Agate soybean, which flowers as promptly as Biloxi if subjected to rather short photoperiods, cannot be prevented from flowering by the use of any amount of artificial light applied as a supplement to the natural illumination. Its flowering can be somewhat delayed but even with continuous light, consisting of sunshine during the daytime and 2000 foot-candles of artificial light at night, the production of flowers occurs relatively early. Such a type of plant should therefore be avoided if the investigator wishes to conduct his work with a vegetative test plant.

If very early flowering of a test plant such as soybean were desired, either of the two varieties discussed would prove satisfactory insofar as their photoperiodic responses are concerned. For such a purpose it would be necessary to reduce the length of daily photoperiod to 8 or 10 hours by means of light-tight covers over the plants. With such reduced day-lengths, seedlings of both varieties initiate their first flower buds in the axil of the second or third compound leaf, and they flower when about 4 weeks old. The chief requirement of covers made for this purpose is that they exclude all light, particularly if plants of the Biloxi type are used, because light intensities as low as 0.01 foot-candle continued throughout the "dark period" will prevent flowering of this plant. This fact and the effect of brief periods of somewhat stronger light near the middle of the dark periods serve to emphasize the importance of having complete and uninterrupted darkness following each short photoperiod. Such conditions are not difficult to attain, however, and if rigidly adhered to will ensure prompt flowering of the test plants.

With test plants that exhibit long-day responses, the same general principles apply. In certain types it is probable that varieties may be selected that are only moderately sensitive to the range of natural photoperiods encountered. If such varieties are unavailable, artificial manipulation of the photoperiod may

be advantageously practiced. With long-day plants, reduction in length of photoperiod below the critical length promotes vegetative development, whereas extension of the photoperiod above the critical makes possible or hastens reproductive development. The intensity of supplemental light required to accelerate flowering of many long-day plants is reported to be somewhat greater than that required to prevent flowering of the short-day Biloxi soybean. With most long-day plants, however, intensities of 100 foot-candles or even less are reported to produce rather striking accelerative effects on flowering.

Many of the cereals such as barley and wheat, which are frequently used as test plants in soil studies, are of the long-day type. Varieties of these plants differ in their sensitivity to photoperiod and this makes it possible for an investigator to select types that are best adapted to his needs. Although detailed descriptive data of the photoperiodic responses of numerous varieties of cereals are not yet available, the spring varieties are reported to be more sensitive to photoperiod than are winter ones (6). The development of most varieties can be strongly influenced by the use of artificial light of low intensity during periods of short natural photoperiod. Winter barley, for example, grown with 12-hour photoperiods of natural light produces a prostrate, rosette type of growth and fails to develop normal heads. The addition of 3 hours of Mazda light having an intensity of about 40 foot-candles results in erect growth and prompt heading. Such effects are remarkable in view of the fact that the energy received from the 3 hours of artificial light is negligible in comparison to that received during the 12 hours of natural light. Thus with plants of this type the investigator can either select varieties that most nearly meet his requirements when they are grown under prevailing light conditions, or he can modify the daily duration of light to suit the requirements of his experimental material. The latter course affords him a greater choice among available varieties and probably much closer control of development as well.

The preceding discussion has been concerned mainly with photoperiodically induced differences in the reproductive condition of plants because such differences are the type probably most commonly encountered by those who conduct greenhouse experiments throughout different seasons of the year. Other phases of plant development such as bulb formation are modified by photoperiod, however, and for the purpose of some soil experiments test plants having such types of photoperiodic responses might be necessary. The onion is such a plant (7). The bulbing of onions, which is influenced by a number of environmental factors, can be rather accurately controlled by regulation of photoperiod, when other factors are not limiting. Long photoperiods promote bulbing, and short ones delay or completely prevent it.

Onion varieties differ widely in the critical day-length at and above which bulbing begins, but nearly all can be induced to bulb very early if they receive sufficient supplemental light. Temperature and other environmental factors also play a part, but if these factors are within the range commonly available in greenhouse culture, control of photoperiod can be employed to regulate bulbing.

Many varieties of onion can be induced to bulb when they have only one or two leaves if they are supplied with only a few hours of supplemental low-intensity light daily. An experimenter can thus work with test plants that are in the process of bulb formation or with those that are still actively producing new structures and that have no tendency toward storing a reserve of food. The nutrient intake of plants of these two types from the soil or culture solution in which they are grown is probably different, and for this reason the experimenter might find it desirable to conduct comparative tests. By utilizing the photoperiodic response of onions, he can have material of a single variety and age in either or both developmental conditions when the seedlings are only a few weeks old or at any later time that he may choose.

If regulation of plant development is to be attempted through photoperiodic manipulation, control of photoperiod in most cases should be maintained from the time the seedlings appear above ground. With many plants very few repetitions of certain photoperiods may induce permanent changes which the investigator desires to avoid. A single cycle of short day and long night in the case of cockle bur (5) and two such cycles in the case of Biloxi soybean (2) have been reported to induce flowering in these plants. Flower primordia have been observed on barley seedlings approximately 10 days after the seeds were planted, provided the seedlings received long photoperiods as soon as they emerged from the soil (3). Four to six cycles of short photoperiods are sufficient to induce flowering in hemp plants. With these plants and many others, a week of photoperiods favorable to flowering may be more than sufficient to induce a condition of reproductive development from which a plant cannot be reversed by any amount of photoperiodic treatment subsequently applied. Therefore, artificial control of photoperiod should begin at the time of planting unless it is known that the natural photoperiods occurring at the time are favorable to the type of development desired.

#### METHODS AND EQUIPMENT FOR CONTROL OF PHOTOPERIOD

Regulation of photoperiod presents different problems, depending upon the length of photoperiod desired. If it is necessary to reduce the photoperiod to less than the natural day-length, precautions must be taken to avoid increased temperatures resulting from the covers placed over the plants. These precautions include choice of time for covering and uncovering the plants and proper design of covers.

Much of the heat can be avoided if the natural dark period is lengthened by approximately equal amounts, night and morning. This is easily done by placing the covers over the plants at the desired time in the afternoon, leaving them in position overnight, and removing them at the proper time next morning. This results in exclusion of equal periods of light night and morning when the heating effect of the sun is least. For this reason, it is a better practice than covering for a longer period at either the beginning or end of the natural day. In addition to reducing heat effects, this procedure has the added advantage of making the length of the effective photoperiod accurately measurable. If the photoperiod begins or ends with twilight, the intensity of which fluctuates with

the time of day, it becomes nearly impossible to know the time when the light intensity reaches a level that begins or ceases to be photoperiodically effective. It is therefore impossible to state accurately the length of photoperiod employed.

To shorten the photoperiod for plants maintained on conventional greenhouse benches, frames may be erected over the benches and upon them one or more thicknesses of black sateen may be spread to exclude the light (fig. 1). This cloth comes in various grades, some of which are much more impervious to light than others. A quality should be selected that in full sunlight will transmit less

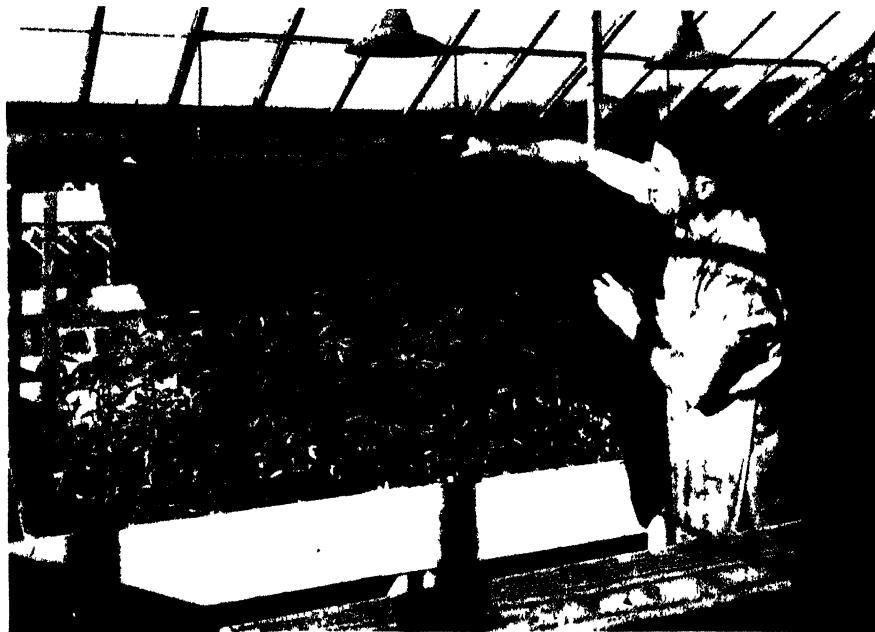


FIG. 1. HEMP PLANTS RECEIVING CONTROLLED PHOTOPERIODS ON A GREENHOUSE BENCH

The covers consist of a double layer of black sateen stitched into a box like structure that fits rather loosely over the supporting frames.

than 0.1 foot-candle through two layers. A layer of white sheeting is sometimes substituted for the outer layer of black cloth because the white material reflects considerable heat. Ventilation of the cages can be effected by making the frames slightly wider than the benches and allowing the cloth to hang somewhat below the bottom of the bench. This leaves a space for air circulation but still excludes the light. For some types of work it is desirable to set up the experiments on warehouse platform trucks which can be moved from the greenhouse into well-ventilated dark chambers in an adjoining headhouse (fig. 2). This system is particularly desirable if very short days are to be maintained, because it is usually easier to control temperature in a permanent forced-draft-ventilated room than in a small cloth cage in the greenhouse.

Lengthening of the photoperiod is easily accomplished by the use of incandes-

cent-filament lamps. With many kinds of plants, 100-watt lamps with 12- to 14-inch reflectors spaced along the middle of the greenhouse bench 3 to 4 feet apart and 2 to 3 feet above the leaf surfaces produce sufficient light to make pronounced differences in development. Light intensity at the surface of pots on such a bench will vary from approximately 25 to 50 foot-candles. Other kinds of lamps such as the fluorescent type now available are also satisfactory for extending the photoperiod. The lamps may be turned on and off by inexpensive electric time-switches, thus making regulation of the photoperiod automatic. These switches are available in a number of types. The one selected should be

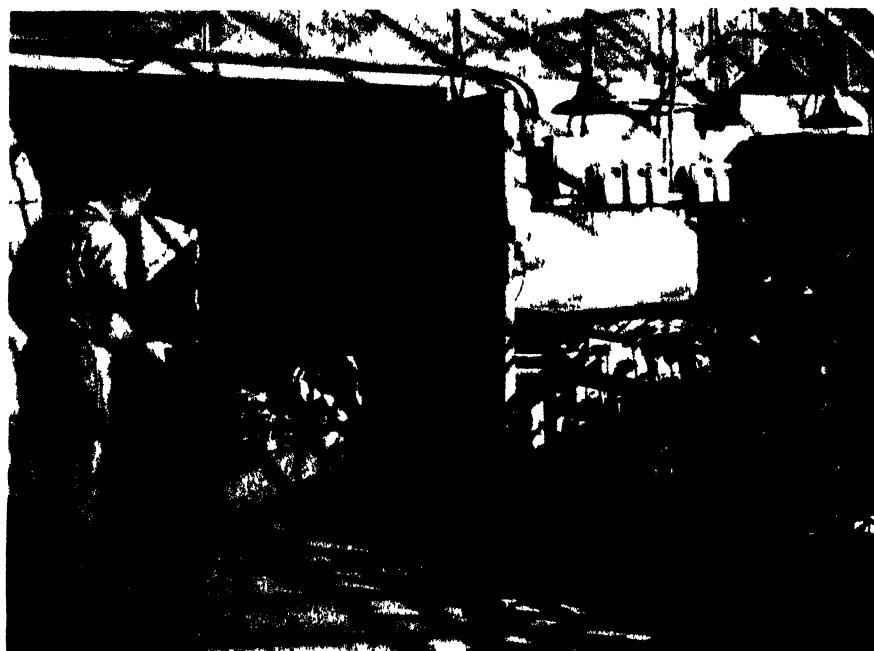


FIG. 2 CASTOR BEANS RECEIVING CONTROLLED DARK PERIODS IN A PERMANENT DARK ROOM ADJOINING THE GREENHOUSE

The dark-room is subdivided into a number of chambers so that plants moved into the dark early will not be illuminated later when the doors are opened to admit other lots.

capable of opening and closing a circuit several times daily and should be designed so that it can be made to open a circuit within a few minutes after it is closed.

#### DISCUSSION

Considerable attention has been given to the fact that the growth and development of photoperiodically sensitive plants can be regulated to considerable degree by comparatively simple methods of controlling day-length. The equipment for such control need be neither complex nor expensive, yet it can in many cases be made automatic and precise in its action. Experimenters regulate

greenhouse temperatures with a reasonable degree of precision as a matter of routine, and they attempt to regulate such factors as humidity, soil moisture, and others which are more difficult to control in the greenhouse. Control of photoperiod is much more simply and accurately attainable. It can be done automatically in many cases by means of an electric time-switch activating a series of lights. Considerable variation in light intensity is without effect so long as the minimum intensity is above a predetermined threshold value. Time of application and duration of the supplemental light are the critical factors. The advantages resulting from such control are, in many cases, fully as great as those resulting from control of other factors and, in some cases, greater.

Selection of a test plant should be based on consideration of all factors that affect its adaptability to a particular experiment. If the plant selected is not photoperiodically sensitive, this factor needs no further attention. If it is sensitive, its response probably can be regulated by artificial control of the photoperiod. It would seem undesirable in most cases to discard an otherwise desirable test plant because of its photoperiodic response until it has been clearly demonstrated that this response cannot be easily controlled by manipulation of daylength.

Many workers have shown that plants accumulate materials from the soil at varying rates throughout the season. These rates are probably more closely correlated with the type of development through which the plant is passing at a particular time than with the actual age of the plant. Considerable emphasis, therefore, is placed on the advantages resulting from the use of test plants the development of which can be rather sensitively regulated by photoperiodic control. If facilities for photoperiodic control can be provided, such plants are more to be sought than avoided for test purposes because of the additional information and more dependable results that can be obtained with suitable experimental procedures.

#### REFERENCES

- (1) ALLARD, H. A., AND ZAUMAYER, W. J. 1944 Responses of beans (*Phaseolus*) and other legumes to length of day. U. S. Dept. Agr. Tech. Bul. 867.
- (2) BORTHWICK, H. A., AND PARKER, M. W. 1938 Influence of photoperiods upon the differentiation of meristems and the blossoming of Biloxi soybeans. *Bot. Gaz.* 99: 825-839.
- (3) BORTHWICK, H. A., PARKER, M. W., AND HEINZE, P. H. 1941 Effect of photoperiod and temperature on development of barley. *Bot. Gaz.* 103: 326-341.
- (4) GARNEE, W. W., AND ALLARD, H. A. 1920 Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. *Jour. Agr. Res.* 18: 553-606.
- (5) HAMNER, K. C., AND BONNER, J. 1938 Photoperiodism in relation to hormones as factors in floral initiation and development. *Bot. Gaz.* 100: 388-431.
- (6) HURD-KARRER, A. M. 1933 Comparative responses of a spring and winter wheat to day length and temperature. *Jour. Agr. Res.* 46: 867-888.
- (7) MAGRUDER, R., AND ALLARD, H. A. 1937 Bulb formation in some American and European varieties of onions as affected by length of day. *Jour. Agr. Res.* 54: 719-752.



# ENVIRONMENTAL FACTORS AND THEIR CONTROL IN PLANT EXPERIMENTS

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The results of a study of plant responses depend to a large degree on the environment to which the plants have been subjected preceding and during the treatment. The factors which are known to exert a great influence are light, temperature, moisture, substrate, and the composition of the surrounding atmosphere. Failure to establish the correct environment or to understand the environment can result in faulty evaluation of results in many types of experimentation. This is especially likely to be misleading when plants are being used in soil fertility or nutrient solution investigations. Many of the conflicting results that have been published on such subjects can be attributed to uncontrolled environments or to lack of knowledge about them. As an example, soybeans grown on the short natural photoperiods prevailing during the winter months will flower and set fruit shortly after emerging from the soil. Another planting made during the longer photoperiods prevailing in the spring months would remain vegetative much longer. If the yields of tops or seeds from these two plantings were used to compare the fertility of different groups of soils or the quality of a series of nutrient solutions, the results would have but limited value, since the change in the photoperiod was the dominant factor causing the difference in yield. Barley is another plant for which the photoperiod must be controlled if successive plantings are to be compared, since it stools out on short photoperiods, and heads readily on long photoperiods. These are but two examples of many plants that respond in a similar manner.

Various influences of environment on growth are, of course, universally recognized, and there are numerous publications in this field. Wilson (15) has published an extensive bibliography on environment in relation to plant diseases. Within the past decade improved equipment has made the control of environmental factors more feasible, and this brief discussion will cover some types of the apparatus employed.

## CONTROLLED ENVIRONMENT ROOMS

The early work in the field of closely controlled environments was confined chiefly to the control of temperature of the root system. This work was done by Jones (6) and his co-workers, and the apparatus they devised with its many subsequent modifications has developed into a more or less standard type known as the "Wisconsin soil temperature tank." These tanks have been extremely valuable in many types of research, but numerous investigators have desired to control additional factors of the environment simultaneously. Consequently,

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several types of control cabinets have been devised to meet specific needs. Most of these control substrate temperature, air temperature, and humidity, and a few also control light. Popp (10) has reviewed the work dealing with the effect of artificial light on plant growth prior to 1924. In general, the work up to that time was conducted under greatly varying conditions, making it very difficult to ascribe an effect to any one factor.

In 1924 the Boyce Thompson Institute of Plant Research built a number of very elaborate controlled-environment greenhouses and constant condition rooms (2). The latter rooms were equipped for growing plants entirely with artificial light. One of these was a light room and the other a dark room. The main source of illumination in the light room was twenty-five 1500-watt gas-filled tungsten-filament lamps, fitted with reflectors to give uniform illumination on the benches where the plants were grown. The lamps were separated from the main room by a false ceiling of clear plate glass over which a  $\frac{1}{2}$ - to  $\frac{1}{4}$ -inch depth of water could be flowed. This glass-water filter absorbed some of the infrared of the lamps and thus kept a great amount of undesired heat out of the room but it absorbed a considerable amount of energy in the visible part of the spectrum, reducing the efficiency of the lighting system.

Arthur, Guthrie, and Newell (1) made a very comprehensive study of plant growth in the constant condition rooms and in the controlled-environment greenhouse. They found that sunlight was a better light source for plant growth than was the incandescent-filament lamp. Their experiments on supplementing the incandescent-filament lamps with white-flame carbon arcs and with mercury-vapor arcs did not result in a visible benefit to the plants grown in the constant condition light room.

There have been many advancements in equipment for controlling the environment since 1924, and in the last few years several papers dealing with controlled environment for plant growth have appeared. The growth chambers described in these papers cover a rather wide range of complexity, and only representative types will be discussed.

Davis and Hoagland (3) in 1928 described an apparatus in which they grew plants under controlled light, temperature, humidity, and culture solution. This chamber was one of the first of its type used for plant growth studies, and in it the environment could be controlled over a wide range and each condition duplicated at will. This fact made it possible to duplicate yields and other external evidences of growth at any time during the year. The illumination for this early chamber, provided by Mazda C gas-filled lamps, permitted the growing of good wheat plants, but the quality of light was not the best for growth of various other types of plants, probably because it contained too little energy in the blue region of the spectrum.

With the advent of fluorescent lamps the illumination of such controlled chambers was greatly facilitated, since this type of lamp produces less heat for a given amount of illumination and has better spectral quality than tungsten-filament bulbs. Naylor and Gerner (8) have shown that the "daylight" and white types of fluorescent lamps can be used as the sole source of illumination

for several species of plants. They pointed out that Hoagland and Went had also been successfully using fluorescent lamps to grow plants.

Hartmann and McKinnon (5) have described the construction and operation of four air-conditioned cabinets which are 3 feet wide, 4 feet long, and 5 feet high. These cabinets control light and temperature and have been used to study the interrelation of temperature and photoperiod in the growth and development of plants. Each cabinet is illuminated with twelve 40-watt, 48-inch fluorescent tubes, a daylight type alternating with a white type. This arrangement gives approximately 500 foot-candles intensity at a distance of approximately 1 foot from the lamps, and the quality approaches the spectral distribution requirements for photosynthesis. The authors asserted that strawberries in the cabinets maintained a growth rate comparable to that of plants grown in the field in the same soil.

Hamner (4) also has recently described a chamber for growing plants under controlled conditions of illumination, humidity, and temperature. An area 6 feet by 8 feet is illuminated with one hundred forty-four 30-watt fluorescent lamps, with daylight and white tubes arranged alternately as in the cabinets of Hartmann and McKinnon to provide the desired quality of light. Hamner reported an intensity of 1000 foot-candles at a distance of 3 feet from the floor and 3.5 feet from the lamps. Though this intensity may be a limiting factor in some types of experiments and with some plants, Hamner reported that several species had been grown from seed to maturity satisfactorily. Temperature is controlled in this chamber by taking advantage of the prevailing climate at Ithaca, New York. Air from the outside is brought in by a fan which is thermostatically controlled. This is an excellent arrangement and saves the cost of a refrigeration plant when the prevailing temperature is sufficiently low to allow operation for an extended period during the year.

At the Bureau of Plant Industry Station, Beltsville, Maryland, four environmental control rooms have been in use since 1937 (fig. 1). These rooms have been modified from time to time and the present equipment has proved very satisfactory for obtaining reproducible growth at any time during the year.

Light, temperature, and humidity are controlled. Each room is constructed as a standard refrigeration room approximately 18 feet by 9 feet by 10 feet. The primary light source in each room is an alternating current arc lamp. At first, the lamps in two of these rooms were operated at 60 amperes across the arc, and those in the other two rooms at 75 amperes. The higher amperage gave an increase in light intensity of about 500 foot-candles at the level of the pots. Although the plants grown in the rooms with the 60-ampere lamps were of very good quality, those grown with the 75-ampere lamps were more sturdy and produced a greater amount of dry weight in a given time. Consequently the 60-ampere lamps were converted to burn at 75 amperes. Each lamp is equipped with a spun-aluminum parabolic reflector which gives a 6-foot band of relatively uniform light around the lamp. "Sunshine" carbons are burned, and the quality of the light approaches sunlight more closely than that of any other single artificial source. The arc is enclosed by a window-glass housing which filters out the

ultraviolet radiation below 3100 Å. In addition to the arc light each room has eight 200-watt incandescent lamps over the plant benches. These lamps were installed after several crops of soybeans which had been grown with only arc light were found to have a carbohydrate content that was low in comparison to that of greenhouse-grown or field-grown soybeans. Since the "Sunshine" carbon spectrum is low in the red region, and that of the incandescent lamps is high, the addition of only a small amount of the latter light made it possible to approach sunlight more closely and to increase photosynthesis. The intensity of the arc



FIG. 1. ONE OF THE ENVIRONMENTAL CONTROL ROOMS AT THE BUREAU OF PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND

The principal source of light is an alternating current arc lamp operating at 50 volts and 75 amperes. This is supplemented with eight 200 watt incandescent lamps. The Biloxi soybeans shown were photographed 3 weeks after the seeds were planted. They were grown in sand culture and received 16 hours of the above light daily. When the plants were harvested at the end of 4 weeks their average height was 28.6 cm. and their average fresh and dry weights per plant were 16.3 gm. and 2.8 gm. respectively.

lamp at a distance of 1 meter was 2000 foot-candles, and that of the incandescent lamps at the same distance was 160 foot-candles, yet the carbohydrate content of the leaves of soybean plants was increased by approximately 100 per cent after the few incandescent lamps were installed.

The arc lamp was installed in the center of each room at a height that made the flame of the arc approximately 6 feet from the floor. Two semicircular benches with three levels were constructed around the lamp to hold the culture crocks. The three levels were arranged so that a relatively uniform intensity of light would fall on all of the plants in the culture crocks. The benches have a diameter of 9 feet and are 3 feet wide. The area directly beneath the lamp is com-

pletely shaded and unsatisfactory for plant growth. Such an arrangement made it possible to have fifty-eight 2-gallon crocks in each room. Since each crock will support four soybean plants, 232 plants can be grown in each room.

Sand culture has been employed extensively in these chambers. The nutrient used is a modified Shive four-salt solution. Experiments with standard solutions did not produce normal plants. Consequently, several modifications in the nutrient had to be made to suit the particular environment of these control rooms. This requirement will unquestionably be found to hold true for other controlled environmental set ups; therefore, when nutrient solutions are employed, the investigator should determine the ionic concentrations permitting maximum plant growth in such artificially regulated environment. A nutrient solution may be in perfect balance for growth under greenhouse conditions but be unsuitable for producing normal plants in control chambers because of limited light intensity.

Temperature is controlled in the rooms by a brine refrigeration system. The brine is cooled by ammonia compressors, and the refrigerant is then pumped to unit coolers suspended from the ceiling and equipped with electric fans for circulating the air of the room through the coolers. The flow of brine to the unit cooler in each room is controlled by a solenoid valve which, in turn, is activated by a mercury-air thermostat.

A stream of air is passed continuously through the arc lamp housing from a semitight duct and is taken away from the lamp through a gas-tight duct. This arrangement allows the gradual introduction of some fresh air and provides facilities to take off all the toxic fumes from the arc light.

Humidity is controlled by means of a single-atomizing spray, which is activated by a humidistat operating a solenoid valve. Water is fed to the spray by gravity, and compressed air is used to atomize the water into the air stream of the fan on the unit cooler. Relative humidity can be controlled over the range of 50 to 100 per cent within 10 per cent. This has been suitable for the culture of soybeans.

The operation of the equipment in these rooms is automatic except for carbon changes. Light is controlled by time switches, temperature by mercury-air thermostats, and humidity by a hair humidistat. In these control chambers soybean plants can be grown that are equal or superior to greenhouse-grown plants on the basis of dry weight, pigmentation, total nodes produced, and chemical composition.

Plant growth chambers that depend on an artificial source of illumination are limited by quality and intensity of the light because no artificial source has yet been developed that is the equal of sunlight in either intensity or quality. Though this is a recognized disadvantage, the use of artificial light is necessary to obtain uniform growth conditions during every season of the year in most parts of the United States. Some sections, however, have more nearly uniform daily exposures of sunlight than others and there it is possible to air-condition a specially constructed greenhouse or glass cabinet and use the sun as the source of light.

Went (13) has recently described the air-conditioned greenhouses at the Cal-

ifornia Institute of Technology. Two independent units, each consisting of a greenhouse and a dark room, are air-conditioned by two separate systems, so that different conditions can be maintained independently in the two units. The control of both temperature and humidity in the units is very precise and is possible over a rather wide range since one unit is equipped with a refrigeration system. Panels of fluorescent lamps are installed in the dark rooms and produce an intensity of 400 to 450 foot-candles 1 foot below the lamps. Although this intensity will enable growth of some plants to maturity, it is approaching the lower limit for most seed-bearing plants and a higher intensity would be advantageous.

Each of the various types of controlled-environment equipment described has its advantages for plant physiological work. Some types of problems demand much more elaborate equipment than others. Since the degree of control required determines in large measure the cost of installation and maintenance, elaborate installations are not always available. Under such circumstances the requirements of problems undertaken should not exceed the potentialities of the equipment.

As the environmental control becomes more refined, the area available for growing plants becomes smaller. With some problems it is better to tolerate less exact control in order that larger populations may be grown. In such cases, however, one should still exercise as much control over the environment as is feasible and should evaluate the results accordingly.

#### LIGHT

The duration of light, or the photoperiod, to which plants are exposed may greatly influence vegetative or floral development. This factor alone must be carefully controlled or successive plantings under natural photoperiod may be more strongly influenced by this factor than by any other being varied. Some plants are not affected by the photoperiod to which they are exposed. Generally speaking, an investigator should know whether or not the plant used is photoperiodically sensitive, and if it is, the photoperiod should be controlled to produce the type of growth desired.

In studying responses to light the question of the type of light naturally comes into consideration. When sunlight is being employed as the principal source in photoperiod studies, the day-length can be extended by low intensity incandescent light. The intensity of this light need not be over 30 to 50 foot-candles. When plants are being grown with artificial light as the sole source of energy, however, the type of light employed must be chosen so that the spectral distribution and intensity will approach that required for photosynthesis.

The standard incandescent-filament lamp has proved unsatisfactory when used as the only source of light energy for growing plants because both the quality of the light emitted and the intensities that can be readily obtained are inadequate. The proper quality of light can be obtained from an arc light burning cored carbons, and from fluorescent lamps. An arc lamp burning "Sunshine" carbons gives a closer approach to sunlight, both in quality and in intensity of light, than

do fluorescent lamps. Nevertheless, plants can be grown with fluorescent light, and the installation and maintenance are somewhat less costly than for arc light. An arc lamp produces considerable heat, which necessitates a refrigeration system of large capacity if temperature is to be accurately controlled, while fluorescent lamps produce relatively less heat and require less refrigeration to control the temperature, particularly if the installation is such that only the lamp tubes are in the room and the auxillary equipment is outside. Arc lamps also require carbon changes every 8 hours, while fluorescent lamps require little attention. Another disadvantage of arc lamps is the fumes that are generated during burning. These fumes are very toxic to plants and must be removed continuously and thoroughly when the lamp is operating. Consequently an investigator should consider the advantages and disadvantages of the sources available and select the one best suited to his problem and to the conditions under which he must work.

#### TEMPERATURE

The early work on environmental control dealt primarily with the effect of temperature on growth rate. It was taught that plants have three cardinal points in relation to temperature: the minimum, the lowest temperature at which growth occurs; the optimum, the temperature at which growth is the greatest; and the maximum, the highest temperature at which growth occurs. Attempts to establish definite optimum temperatures for growth of different plants resulted in conflicting reports because different investigators measured different organs, tissues, or varieties of plants and the effects of other environmental factors were not recognized. Further research has shown that the optimum temperature varies with the age of the plant tested; the light intensity, quality, and duration; available moisture; and nutrition, as well as numerous other environmental factors. The optimum temperature for growth must, therefore, be determined experimentally by the individual for the specific conditions under which the plants are being grown.

The effect of temperature on flowering and fruit formation has also been extensively investigated. As in the case of growth, the results reported have been conflicting because of the influence of other uncontrolled environmental factors. Thompson (12) suggests that in considering the relation of temperature to type of growth, one should distinguish between its effect on the initiation of flowering and its effect on the development of flowers, seed, or fruit. These different physiological stages of development can have distinctly different optimum temperatures, and these can be greatly influenced by the duration of treatment and the conditions following treatment. Celery, beet, and cabbage plants which require a relatively low temperature of 40° to 50°F. or 50° to 60°F. to induce floral initiation, will flower and set seed more rapidly if the temperature is raised to 60° to 70°F. following thermoinduction. If the temperature, however, is raised to 70° to 80°F. following the low-temperature induction period, the plants will remain vegetative indefinitely when grown under the natural photo-period.

The effectiveness of a photoperiod that may result in flowering is influenced by temperature. For example, Biloxi soybean on a short photoperiod and at temperatures above 60°F. flowers promptly, but when held at 55°F. it fails to initiate flowers (9).

Went (14) has recently shown the importance of night temperature upon stem elongation, root development, and fruiting in the tomato. Alternation of temperatures gave increased growth, compared with growth of plants kept constantly at one temperature. Tomato plants kept warm during the day (26.5°C.) and cool during the night (17° to 20°C.) grew more rapidly than when subjected to any of the other constant or alternating temperatures tested. Went (14) proposes the name *thermoperiodicity* for all effects resulting from a temperature difference during light and dark periods on the plant, whether they be vegetative growth, flowering, or fruiting responses. The importance of temperature during the dark part of the cycle has also been shown in photoperiodism. Floral initiation is greatly inhibited in Biloxi soybeans by a low temperature (55°F.) during the dark period (9). This does not imply that the temperature during the light part of the daily cycle is unimportant; it simply means that during the dark part of the cycle reactions in plants may have different optimums than during the light part.

Exact control of temperature in a standard greenhouse during the day is very difficult because of fluctuating radiation from the sun. During the summer months the range of control is limited because of the tremendous amounts of heat that enter as sunlight and that must be removed to obtain even moderate temperatures on bright days. Night temperatures during much of the year are more readily controlled than day temperatures. The best that an investigator can do under these conditions, after determining the range of day and night temperature that can be maintained throughout the season, is to select a test plant tolerant of such a range or, if choice of plant is not possible, to restrict the experimental period to a favorable season.

The usual difficulty encountered in a greenhouse in summer is excessive heat during the day. This can be lowered by shading with paint or whitewash on the glass or by running a sheet of water over the entire roof. Shading has the great disadvantage of reducing the visible-light intensity too much. Running water over the glass does not greatly affect the intensity of visible rays, and it will lower the temperature inside a house by 5° to 10°F., depending on outside atmospheric conditions. This system is also more flexible than shading, since the water can be cut off when cooling is unnecessary. Moreover, if the water is recirculated over the roof the system can be operated rather economically.

Night temperatures can be controlled easily in greenhouses, provided the prevailing temperature outside is lower than that desired inside the house. The usual type of greenhouse pipe-coil radiators can be supplemented with unit heaters equipped with thermostatically controlled electric fans. The radiators can be set manually so that they hold the temperature a few degrees below that desired and the unit heaters will supply the additional heat with good control. It should be realized, however, that the unit heaters equipped with fans produce a

marked turbulence of air throughout the greenhouse, and in some types of pathological work their use may be undesirable for one reason or another. In other types of work it might be an advantage.

In some regions the prevailing climatic fluctuations are small enough over extended periods during the winter months to permit the maintenance of low temperatures during the day in a standard greenhouse. Under these conditions a range of temperatures can be established in different houses or sections of houses and a large number of plants can be given different temperature treatments in a single experiment. Light intensity, during the months when low temperatures are prevalent, is at a minimum for the region, and this factor can influence the response of the plants to the different temperatures. For example, a low light intensity with a low temperature during the light part of the daily cycle limits carbohydrate accumulation and if this is followed by a warm dark cycle the rate of respiration is high and the available food supply is rapidly exhausted. Plants fail to grow under these conditions and on continued treatment usually die. The environmental factors of light and temperature are so closely interrelated that one must be cautious in ascribing a specific effect to either factor.

#### COMPOSITION OF ATMOSPHERE

The composition of the gaseous environment cannot be controlled even within moderate limits without specially constructed equipment. It is generally assumed that the CO<sub>2</sub> content of the air in a greenhouse is as constant as the CO<sub>2</sub> content of the air outside, but this is not always the case. On a bright sunny day the CO<sub>2</sub> content of the air in a greenhouse at Beltsville varied from 0.01 per cent to 0.04 per cent. The lower percentage of CO<sub>2</sub> in the air occurred immediately after the plants were watered and the walks and walls drenched. A half hour later the concentration of CO<sub>2</sub> was still about 0.01 per cent, but at the end of an hour it had increased to 0.03 per cent. This fluctuation was evidently associated with the solution of CO<sub>2</sub> in water and its subsequent release from solution as the temperature of the water increased and as the water evaporated. The CO<sub>2</sub> content of the air in a greenhouse may thus become limiting to growth for short periods, but with adequate ventilation this problem is not serious. Many investigators have studied the effects of increasing the CO<sub>2</sub> content of the air on plant growth, and their results have been summarized by Miller (7, pp. 580-587). In general, growth of plants is increased when the CO<sub>2</sub> content of the atmosphere is increased moderately up to 0.3 per cent unless light or some other environmental factor is limiting. Maintaining an increased CO<sub>2</sub> concentration in a greenhouse is difficult and costly, and therefore it has not been used extensively. Experiments in which control of the gaseous environment is necessary are difficult because the normal atmosphere must be excluded from the plants to permit control. Thomas *et al.* (11) have described a series of greenhouses which consist of large sand-culture beds surmounted by airtight individual greenhouses. These are supplied with washed and filtered air of known composition. These chambers have been used to determine the effect of sulfate and sul-

fur dioxide on plant growth, but they can be used to study the effect of any constituent of the gaseous environment.

#### HUMIDITY

The control of humidity in a greenhouse is usually dependent on the outside atmospheric conditions. Such practices as keeping plants either on the "dry" or "wet" side and wetting the walks and walls are usually resorted to for controlling humidity. Many different systems for humidification have been used in controlled-environment rooms. One type of system most generally employed provides some means of delivering to the air a fine mist of water which quickly vaporizes when the relative humidity falls below a predetermined point. More elaborate air-conditioning units can supply a fairly constant relative humidity, since they are capable of dehumidifying as well as humidifying the air.

Dehumidifying equipment is generally expensive to install and to maintain in plant-growing chambers. Such systems depend either upon refrigeration to condense part of the moisture of the air or upon such dehydrating agents as silica gel, calcium chloride, or triethylene glycol to absorb it. Any of these devices involves rather elaborate apparatus and considerable expenditures.

Went (14) showed that the growth rate of the main stem of the tomato plant was not dependent upon the relative humidity of the air when this was varied from 45 per cent to 75 per cent. Growth rate increased temporarily when the plants were transferred from low to high humidity and decreased when the transfer was from high to low, but following these transitory changes the original growth rate was resumed. Since the relative humidities which Went used were not extreme, his conclusions as to the effect of humidity on growth must be restricted to the range tested.

#### DISCUSSION

Of the various environmental factors that affect the growth of plants in greenhouses, the light duration, or photoperiod, and such conditions of the soil as temperature, moisture, aeration, reaction, and nutrients can readily be controlled. The factors of air temperature, humidity, and composition are the most difficult to control because of the constantly and widely changing outside environment. If these factors are to be controlled within a narrow range, air-conditioning of a greenhouse or construction of controlled-environment rooms is necessary. The installations do not have to be excessively elaborate or costly. Several have been described (3, 4, 5) which can be constructed and maintained at a cost within reach of many research institutions. The construction of chambers has been greatly facilitated by the development of fluorescent type lamps and the improvements in air-conditioning equipment have made it possible to condition small greenhouses satisfactorily. If the prevailing climate affords ample sunlight for extended periods, small air-conditioned greenhouses are to be recommended for most experiments, since no artificial source of illumination has been developed that is equivalent to sunlight.

The population of plants that can be grown at one time is very much limited by either the small greenhouse or control chamber, but the results of studies

performed under controlled conditions can serve as a guide in selecting the time and location to perform more extensive field or greenhouse experiments. An outstanding example of such application is in the growing of sugar beet seed. Small-scale experiments showed the necessity of a thermoinduction period followed by growth under a long photoperiod. Such conditions were known to exist in the Pacific Northwest area, and large-scale field plantings made in that area were successful.

Plant growth is dependent upon all the factors that make up the environment. No particular level of one factor should be referred to as the optimum for growth of a species without specifying at least the approximate levels or conditions of the other important components of the environment. No single optimum for any factor exists that will hold without regard to the status of other factors. In other words, no single factor in the environment produces its effect simply, but this effect involves interactions of a high order with those produced by other factors. It will be necessary, therefore, to study the interrelationships, the interactions, of all possible factors in the environment before it will be possible to define the optimum "climate" for the growth of a specific plant or organism.

#### REFERENCES

- (1) ARTHUR, J. M., GUTHRIE, J. D., AND NEWELL, J. M. 1930 Some effects of artificial climates on the growth and chemical composition of plants. *Amer. Jour. Bot.* 17: 416-482.
- (2) Contributions from the Boyce Thompson Institute of Plant Research. Organization, Equipment and Dedication. 1925 Vol. 1. no. 1.
- (3) DAVIS, A. R., AND HOAGLAND, D. R. 1928 An apparatus for the growth of plants in a controlled environment. *Plant Physiol.* 3: 277-292.
- (4) HAMNER, K. C. 1944 Description of a chamber for growing plants under controlled conditions. *Bot. Gaz.* 105: 437-441.
- (5) HARTMANN, H. T., AND MCKINNON, L. R. 1943 Environment control cabinets for studying the inter-relation of temperature and photoperiod on the growth and development of plants. *Proc. Amer. Soc. Hort. Sci.* 42: 475-480.
- (6) JONES, L. R., JOHNSON, J., AND DICKSON, J. G. 1926 Wisconsin studies upon the relation of soil temperature to plant disease. *Wis. Agr. Exp. Sta. Res. Bul.* 71.
- (7) MILLER, E. C. 1938 *Plant Physiology*, ed. 2. New York.
- (8) NAYLOR, A. W., AND GERNER, G. 1940 Fluorescent lamps as a source of light for growing plants. *Bot. Gaz.* 101: 715.
- (9) PARKER, M. W., AND BORTHWICK, H. A. 1939 Effect of variation in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean. *Bot. Gaz.* 101: 145-167.
- (10) POPP, H. W. 1924 Summary of literature on some phases of the effect of light on plant growth. *Trans. Illum. Engin. Soc.* 19: 981-994.
- (11) THOMAS, M. D., et al. 1943 An installation of large sand-culture beds surmounted by individual air-conditioned greenhouses. *Plant Physiol.* 18: 334-344.
- (12) THOMPSON, H. C. 1939 Temperature in relation to vegetative and reproductive development in plants. *Proc. Amer. Soc. Hort. Sci.* 37: 672-679.
- (13) WENT, F. W. 1943 Plant growth under controlled conditions: I. The air-conditioned greenhouses at the California Institute of Technology. *Amer. Jour. Bot.* 30: 157-163.
- (14) WENT, F. W. 1944 Plant growth under controlled conditions: II. Thermoperiodicity in growth and fruiting of the tomato. *Amer. Jour. Bot.* 31: 135-150.
- (15) WILSON, J. D. 1932 Environmental factors in relation to plant disease and injury: a bibliography. *Ohio Agr. Exp. Sta. Tech. Ser.* 9.



## OXIDATION OF NITRITES AND OXALATES IN SOILS

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It is now a well-established fact that ammonia and ammonium salts formed in soils by the breakdown of nitrogenous organic matter or added in manures are oxidized to nitrites and nitrates before being assimilated by plants. According to the generally accepted view, this process, known as "nitrification," is biological in nature and is affected in two stages by two distinct species of bacteria.

The rate of nitrification is considered to be greater than that of ammonia production. Ammonia, therefore, does not tend to accumulate, as it is converted into nitrites and nitrates as soon as it is produced from organic matter.

Because of its simplicity and the divergence of existing views regarding its mechanism, the reaction involving the oxidation of nitrites to nitrates by soils was studied in detail in the present work.

This investigation formed a logical sequence to a preliminary study by Puri and Asghar (15) on the oxidation of oxalates to carbonates. The reaction has been shown to be slow, although it tends to reach an end point. It could not be ascribed to any specific substance in the soil but was considered to be quite independent of microbiological activity. This reaction also was studied in detail in the present work.

Rao, Dhar, and their collaborators, in a series of papers (5, 6, 7, 17), have suggested that nitrification in soils must be, in part, photochemical in nature, taking place at the surface of the soil under the influence of sunlight and in the presence of various photosensitizers. They explained the observations of McBeth and Smith (11) and of Lyons (10) regarding the influence of the nature of soil on nitrification reactions as due to varying amounts of these sensitizers present in a soil.

Corbet (3), in a detailed investigation of the subject of nitrification, has confirmed, in large measure, the results of Dhar and Rao and has further shown that some of the stages may be affected by microbiological, chemical, or photochemical agencies. Fraps and Sterges (9), on the other hand, observed no evidence of photonitrification in sterilized soils and, therefore, concluded that photonitrification is of little or no importance in nature.

De Rossi (4) maintained that nitrification in soils takes place by simple physicochemical means. He pointed out that, as in Dhar's experiments, the soil was kept under artificial conditions. It is doubtful whether the results obtained by him could be quoted to explain the natural phenomenon of nitrification in soil.

Moreover, it should be remembered that in Rao and Dhar's original experiments, the formation of nitrates, which are the ultimate product of the nitrifi-

fication process, was not observed. This is because of another photochemical process by which nitrates are converted into nitrites. In later work, however, Dhar and Tandon observed (8) photooxidation of nitrites to nitrates taking place in the presence of various photocatalysts, including the soil. These two reversible photochemical reactions were shown to approach the equilibrium stage after some time. Corbet (2) considered that the conversion of nitrites to nitrates could also take place by a physicochemical process in the presence of H ions. Rao (18), repeating original experiments of Muntz (12), observed conversion of nitrites to nitrates by bubbling CO<sub>2</sub> through nitrite solutions for about 8 hours.

It should be clear from a brief review of the divergent views that despite the vast amount of work done, the problem still lacks conclusive evidence on the exact mechanism of the reactions.

TABLE 1  
*Rate of oxidation of KNO<sub>2</sub> by P.C. 13 acid-treated soil*  
0.212 gm. KNO<sub>2</sub> added to 5 gm. soil

TIME hours	KNO <sub>2</sub> OXIDIZED IN DARK gm.	KNO <sub>2</sub> OXIDIZED IN DIFFUSED LIGHT gm.	KNO <sub>3</sub> FORMED gm.
½	.014	.017	.020
1½	.037	.047	.045
2½	.072	.072	.087
5½	.123	.119	.148
7½	.156	.152	.191
9½	.170	.172	.202
13	.199	.206	.240

#### EXPERIMENTAL

##### *Oxidation of nitrites by soils*

In the preliminary experiments a black cotton soil (P.C. 13) that had been freed of exchangeable bases by prolonged acid treatment was used. Its base-exchange capacity was about 50 m.e. per 100 gm. of soil. To each of a series of 5-gm. portions of the soil, 25 cc. of 0.1 N KNO<sub>2</sub> was added and the volume was made to 100 cc. with water in 500-cc. bottles. The contents were shaken in a mechanical shaker, and the bottles were taken out one by one after various intervals, thus allowing the reaction to take place for different periods in different bottles. The contents were then analyzed for nitrites and nitrates.

In these experiments, as the shaker was kept in the laboratory room, there was no chance for direct sunlight to influence the course of reaction. Another lot of bottles, containing exactly similar quantities of soil and KNO<sub>2</sub> solutions as in previous experiments, were wrapped in thick black papers as a further safeguard against the action of light, if any. These bottles were also withdrawn one by one after different intervals and analyzed for nitrites and nitrates. The results of both experiments are given in table 1.

It is seen that the oxidation of  $\text{KNO}_2$  to  $\text{KNO}_3$  began almost instantaneously and was completed in about 13 hours. Further, it is obvious that the reaction took place quite independently of light; this is clear from the fact that almost equal amounts of nitrites were oxidized in covered and in exposed samples in the same period. It is worth noting that within a brief period of 13 hours, as much as 0.2 gm. of  $\text{KNO}_2$  was completely oxidized to  $\text{KNO}_3$  by only 5 gm. of soil. Incidentally, no bacterial agency could be expected to bring about such huge conversions under normal conditions.

In order to study the oxidation of nitrite solutions of higher concentrations by 5 gm. of soil, increasing amounts of  $\text{KNO}_2$  were added to 5-gm. portions of the soil (P.C. 13 acid-treated), and the volume was made up to 100 cc. in each case. The samples were shaken overnight for 13 hours and analyzed next

TABLE 2  
*Oxidation of varying amounts of  $\text{KNO}_2$  in 13 hours by P.C. 13 acid-treated soil*  
Amount of soil, 5 gm.

$\text{KNO}_2$ ADDED gm.	$\text{KNO}_2$ OXIDIZED		$\text{KNO}_2$ ADDED gm.	$\text{KNO}_2$ OXIDIZED	
	gm.	per cent		gm.	per cent
.0425	.0412	97.0	.4248	.3993	94.0
.1062	.1030	97.0	.5088	.4198	82.5
.2124	.2064	97.2	.8496	.4046	47.6
.3186	.3074	96.5			

TABLE 3  
*Rate of oxidation of  $\text{KNO}_2$  by P.C. 13 acid-treated soil*  
0.8496 gm.  $\text{KNO}_2$  added to 5 gm. soil

TIME	$\text{KNO}_2$ OXIDIZED		TIME	$\text{KNO}_2$ OXIDIZED	
	gm.	per cent		gm.	per cent
13 hours	.4086	48	3 days	.5607	66
1 day	.4250	50	3½ days	.6117	72
2 days	.4842	57	5 days	.7646	90

morning for nitrites. The results, given in table 2, show that the amount of  $\text{KNO}_2$  oxidized increased with the increasing concentration of  $\text{KNO}_2$  added, and that up to 0.42 gm. of  $\text{KNO}_2$  added, the amount oxidized was almost 100 per cent. Beyond this point the percentage oxidation decreased. But as this was brought about by only 5 gm. of the soil and within only 13 hours, it seemed possible that by extending the period, the reaction might be brought to completion.

To test this point, the highest concentration of  $\text{KNO}_2$ , 0.8496 gm. per 100 cc., was shaken with 5 gm. of soil for different lengths of time. The results, given in table 3, show that percentage oxidation continues to increase with time, although the maximum change was brought about within the first 13 hours, during which almost 50 per cent of the total amount was oxidized. The rate of oxidation decreased after this stage.

The effect of different weights of soil on the amounts of nitrites oxidized was determined next. Twenty-five cubic centimeters of 0.1 *N*  $\text{KNO}_2$  solution was added to each of a number of soil samples of increasing weights. The volume was made up to 100 cc. in each case, and the samples were shaken for 13 hours as in the previous experiments. The results are given in table 4 and show that increasing weights of the soil brought about increased oxidation and that in 13 hours about 4 to 5 gm. of soil almost completely oxidized the  $\text{KNO}_2$  added, namely, 0.212 gm.

In 13 hours, 1 gm. of soil oxidized only 0.0524 gm. out of 0.212 gm. originally added. When, however, the reaction was allowed to continue for a longer period

TABLE 4  
*Oxidation of  $\text{KNO}_2$  in 13 hours by varying amounts of P.C. 13 acid-treated soil*  
Amount of  $\text{KNO}_2$  added, 0.212 gm.

WEIGHT OF SOIL gm.	pH AFTER SHAKING	$\text{KNO}_2$ DECOMPOSED gm.	WEIGHT OF SOIL gm.	pH AFTER SHAKING	$\text{KNO}_2$ DECOMPOSED gm.
0	8.2	0	4	2.94	.1884
1	4.26	.0524	5	2.74	.1954
2	3.74	.1274	7	2.34	.2070
3	3.36	.1484	10	2.32	.2084

TABLE 5  
*Rate of oxidation of  $\text{KNO}_2$  by P.C. 13 acid-treated soil*  
0.212 gm.  $\text{KNO}_2$  added to 1 gm. soil

TIME	OXIDATION per cent	TIME	OXIDATION per cent
13 hours	24.7	80 hours	91.7
24 hours	49.8	8 days	95.9
48 hours	81.3	22 days	100.0
60 hours	91.7		

it was found to be progressive, and in about 80 hours, more than 90 per cent of the amount added was oxidized. These results are given in table 5.

To test the effect of mechanical shaking of the suspension on the extent of the reaction, a sample was kept in the mechanical shaker and another sample was kept for the same time without shaking. The results showed that although almost the entire 0.212 gm. of  $\text{KNO}_2$  was oxidized by the mechanically shaken sample in 13 hours, only 0.08 gm. could be oxidized by the other in the same time. The presence of oxygen is essential for this reaction. Some experiments were made in bottles of smaller capacity so that there was very little space for air above the solution. The amounts oxidized were very small and in one case when the bottle was almost completely filled with the solution, no oxidation was observed.

From the various results mentioned, it is evident that oxidation of nitrites to nitrates by soils is always brought to completion in time, depending on the weight of the soil involved. As already mentioned, this reaction takes place quite independently of light and is, therefore, not at all photochemical in nature. Further, it cannot be ascribed to any microbiological factors, as the soil used in the foregoing experiments had been continuously leached with 0.05 *N* HCl in order to free it of exchangeable bases, and it is hardly possible that after this drastic treatment any bacteria would have survived or, having survived, could have shown such colossal activity.

To show conclusively that the reaction takes place quite independently of

TABLE 6  
*Effect of sterilization of soils on oxidation of KNO<sub>2</sub>.*  
Amount of KNO<sub>2</sub> added, 0.212 gm.

SOIL	KNO <sub>2</sub> DECOMPOSED BEFORE STERILIZATION	KNO <sub>2</sub> DECOMPOSED AFTER STERILIZATION
	gm.	gm.
<i>Steam under 50 pounds' pressure in autoclave</i>		
P.C. 13.....	.204	.1997
P.C. 142.....	.2066	.1760
P.C. 161.....	.200	.191
P.C. 246.....	.1422	.1070
Bentonite.....	.1952	.1784
<i>Chloroform</i>		
P.C. 13.....	.212	.1924
P.C. 246.....	.1412	.1401
<i>HgCl<sub>2</sub></i>		
P.C. 13.....	.212	.1924
P.C. 246.....	.1412	.1390
Bentonite.....	.1952	.181

any bacterial action, soil P.C. 13 as well as a few others (all acid-treated) were sterilized in an autoclave under a pressure of 50 pounds before being mixed with nitrite solution. Tests were also made on two soils kept in contact with chloroform for about a week, after which the chloroform was completely evaporated. Other samples were treated with a solution of mercuric chloride and then tested for their oxidation reaction toward KNO<sub>2</sub>. The amounts of nitrite oxidized by sterilized and unsterilized soils are given in table 6. In no case was the extent of the reaction affected by sterilizing the soil. These results leave no doubt that biological factors played no part whatsoever in the oxidation of nitrites to nitrates by soils.

The foregoing experiments were carried out under rather artificial conditions,

a technique adopted because it lent itself to easy reproducibility. To verify the results, further tests were made with very much smaller amounts of water under conditions that prevail in a nitrifying soil.

Five-gram portions of P.C. 284 acid-treated soil having a base-exchange capacity of 20 m.e./100 gm. were each mixed with 2.5 cc. water containing 0.0559 gm. of  $\text{KNO}_3$ , and the amount oxidized was determined after different intervals. In another set of experiments the total volume of solution was made to 100 cc. by adding 97.5 cc. of distilled water, and the amounts of nitrite oxidized after different intervals of time were determined as before. The results, given in table 7, show that oxidation proceeded at a much faster rate when the amount of water was small.

TABLE 7  
*Oxidation of  $\text{KNO}_3$  solution by P.C. 284 acid-treated soil*  
0.0559 gm.  $\text{KNO}_3$  mixed with 5 gm. soil

TIME	$\text{KNO}_3$ OXIDIZED	
	Water added 2.5 cc.	Water added 100 cc.
	gm.	gm.
6 hours	.0351	.0120
24 hours	.0440	.0146
48 hours	.0481	.0160
7 days	.0559	.0165

TABLE 8  
*Oxidation of  $\text{KNO}_3$  by P.C. 284 natural soil*  
0.0559 gm.  $\text{KNO}_3$  added to 5 gm. soil

TIME	$\text{KNO}_3$ OXIDIZED	
	gm.	gm.
24 hours		.0012
48 hours		.0012
7 days		.0051
16 days		.0056

It is clear, therefore, that the general conclusions previously reached are not vitiated by the fact that the experimental conditions under which nitrification was studied were different from those that prevail in a nitrifying soil.

Experiments were next made with soil P.C. 284 in a natural state. Five-gram portions were each mixed with 2.5 cc. of the  $\text{KNO}_3$  solution containing 0.0559 gm. of the salt. The results, given in table 8, show that nitrification in the natural soil, though small, was still appreciable.

Similar experiments were next made with a number of natural soils. Five-gram portions were each kept in contact with 2.5 cc. of solution containing 0.07563 gm. of  $\text{KNO}_3$  for 1 week, after which the amount oxidized was determined in the usual way. The results are given in table 9. There was appre-

ciable nitrification in all the soils. There is a definite indication that the higher the state of saturation of soil, the lower the nitrification.

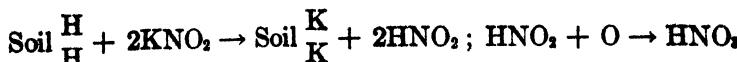
#### *Mechanism of the reaction*

The results of the foregoing experiments show conclusively that oxidation of nitrites to nitrates in soil can take place quite independently of microbiological or photochemical processes. Further, since as much as 0.5 gm. of  $\text{KNO}_2$  can be oxidized by only 5 gm. of soil within a few hours (table 2), it is evident that the reaction is not due to the small amounts of  $\text{CO}_2$  that may be contained in soil or water. Rao (18) could oxidize only about 20 cc. of 0.002 *N* sodium nitrite by bubbling a current of air and  $\text{CO}_2$  through the solution continuously for 8 hours. Our results further show that the reaction is of a continuous nature, as larger and larger amounts of nitrites were oxidized in time, although the amount of the soil remained the same (tables 3 and 4).

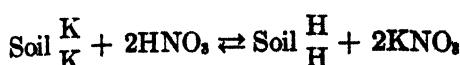
TABLE 9  
*Oxidation of nitrites by natural soils*  
0.07563 gm.  $\text{KNO}_2$  added to 5 gm. soil

SOIL	pH	BASE-SATURATION <i>per cent</i>	KNO <sub>2</sub> OXIDIZED IN 7 DAYS
			gm.
P.C. 1.....	8.45	57	.01483
P.C. 3.....	7.64	73	.01333
P.C. 4.....	9.55	53	.01511
P.C. 5.....	8.77	57	.0089
P.C. 8.....	8.41	66	.0051
P.C. 9.....	5.76	21	.0268
P.C. 10.....	8.71	67	.0049
P.C. 13.....	8.53	80	.0069

The reason for the complete conversion of nitrites to nitrates must lie in the acidoid nature of the soil particles. The soil acidoid on reacting with  $\text{KNO}_2$ , for instance, is converted into potassium soil, thereby liberating  $\text{HNO}_2$ , which as soon as formed is oxidized to  $\text{HNO}_3$  by the oxygen present in the air in the bottle:



The nitric acid then reacts with the remaining  $\text{KNO}_2$ , forming  $\text{KNO}_3$  and liberating fresh  $\text{HNO}_2$  (which again is oxidized to  $\text{HNO}_3$ ), as well as with the potassium soil, changing it back into H-soil. The regenerated H-soil would again react with the remaining  $\text{KNO}_2$ , liberating fresh  $\text{KNO}_3$ , and so on. When after a while there is sufficient accumulation of  $\text{KNO}_3$ , nitric acid will not be able to produce fresh H-soil on account of the reversibility of the reaction.



This explains why the rate of oxidation decreases after some time. But the reaction still continues on account of the displacement, by nitric acid, of  $\text{KNO}_3$ , from the remaining potassium nitrite.

Though Dhar (8) observed oxidation of small amounts of nitrites after exposure to direct sunlight for prolonged hours, extending over several days, the reaction probably was not the one going on in nature. The foregoing physico-chemical explanation is not only fully borne out by the various results described, but is also the simplest and most natural one. This also seems to be the reason why, in nature, nitrates are found in abundance and nitrites only in small quantities.

Ashby (1) has reported that the presence of ammonium salts such as ammonium sulfate inhibits the oxidation of nitrites to nitrates by nitrobacter. Inasmuch as, in nature, the ammonification and nitrification are going on simultaneously, the oxidation of nitrites to nitrates cannot be brought about through the bacterial agency alone.

In order to study the influence, if any, of ammonium sulfate on the physico-chemical oxidation of nitrites, 0.212 gm. of  $\text{KNO}_3$ , dissolved in 100 cc. water was added to 5 gm. of soil containing 0.5 gm. of ammonium sulfate. After being shaken for 13 hours, the sample was analyzed for nitrites. The results were similar to those obtained without the addition of ammonium sulfate, that is, in both cases almost all the  $\text{KNO}_3$  had disappeared. This clearly shows that in nature the ammonium salts cannot influence the reaction.

From the foregoing discussion it is evident that whatever may be the cause of conversion of ammonia to nitrites—the first stage of the nitrification process—the second stage may be completed by a purely physicochemical process.

The oxidation of nitrites to nitrates brought about by different weights of P.C. 13 acid-treated soil was compared with that brought about by corresponding amounts of  $\text{HCl}$  and  $\text{CH}_3\text{COOH}$ . As the base-exchange capacity of this soil is about 50 m.e. per 100 gm., it is evident that 1 gm. of this soil is equivalent to 5 cc. of 0.1 *N* acid. Accordingly, different weights of soils and equivalent amounts of 0.1 *N*  $\text{HCl}$  and 0.1 *N*  $\text{CH}_3\text{COOH}$ , were each kept in contact with 100 cc. of nitrite solution containing 0.212 gm. of  $\text{KNO}_3$ , and the samples were shaken as usual. The results, given in table 10, show that the amounts oxidized by the soil (P.C. 13 acid-treated) lie in between those oxidized by  $\text{HCl}$  and those oxidized by  $\text{CH}_3\text{COOH}$ . It is interesting to note that, beyond a certain concentration, the soil acidoid can oxidize larger amounts than can acetic acid. Similar observations had been made by Puri (13) on the relative powers of acetic acid and the soil acidoid (P.C. 13 acid-treated) in causing inversion of cane sugar.

Further, it is seen that the amounts oxidized by soil compare rather favorably with those oxidized by  $\text{HCl}$ , in view of the enormous difference in the relative strengths of the two types of acids. This is due to the continuous nature of the reaction. The H ions initiate the reaction, and the nitric acid produced carries it further. The amount of nitric acid produced initially would, of course, alter the rate of reaction considerably, and that is the reason why increasing

amounts of soil, as well as of acetic acid and hydrochloric acid, oxidize larger quantities of nitrites in the given time, namely, 13 hours.

Further evidence regarding the influence of acidity on this reaction was obtained by determining the extent of oxidation brought about by P.C. 13 acid-treated soil after gradual neutralization to different pH values. To 5-gm. portions of the soil increasing amounts of 0.1 N NaOH were added and the pH values determined after 48 hours' mechanical shaking when the reaction between soil and alkali was considered to have been completed. The samples

TABLE 10

*Oxidation of KNO<sub>2</sub> by equivalent amounts of soil acidoid, CH<sub>3</sub>COOH, and HCl*  
Amount of KNO<sub>2</sub> added, 0.212 gm.

SOIL ACIDOID, P.C. 13		ACETIC ACID		HYDROCHLORIC ACID	
Soil added gm.	KNO <sub>2</sub> oxidized gm.	0.1 N acid added cc.	KNO <sub>2</sub> oxidized gm.	0.1 N acid added cc.	KNO <sub>2</sub> oxidized gm.
0.5	.010	2.5	.012	2.5	.032
1.0	.0510	5.0	.033	5.0	.095
2.0	.1259	10.0	.063	10.0	.173
3.0	.1501	15.0	.089	15.0	.191
4.0	.1890	20.0	.107	20.0	.194
5.0	.200	25.0	.119	25.0	.199

TABLE 11

*Effect of neutralizing P.C. 13 acid-treated soil with increasing amounts of NaOH on the oxidation of KNO<sub>2</sub>*  
0.212 gm. KNO<sub>2</sub> added to 5 gm. soil

NaOH ADDED cc.	pH AFTER SHAKING WITH NaOH	KNO <sub>2</sub> OXIDIZED gm.
0	3.5	.1954
5	4.5	.1021
10	5.36	.0424
20	5.72	.001
30	6.44	0
40	7.32	0
50	8.72	0

were then mixed with KNO<sub>2</sub> solution and shaken for 13 hours as before. The amounts oxidized in several cases are given in table 11, and are seen to decrease with gradual neutralization of the acidoid. When the pH value reached about 5.7, no oxidation took place in 13 hours. But this only means that at this pH value the rate of reaction diminished and not that it stopped altogether, because when tests were made after long intervals of time with the last two samples, having pH values equal to 7.32 and 8.72 obtained by the addition of either NaOH or Ca(OH)<sub>2</sub> to the acidoid, and also with four natural soils containing all the exchangeable bases originally present and having pH values between

8 and 9, it was found that appreciable amounts of nitrites were oxidized. The nitrite solution kept alone for the same length of time showed no conversion into nitrate. These results, given in table 12, leave no doubt that in nature the residual acidity of the soil is strong enough to bring about the conversion of nitrites into nitrates. It should also be remembered that in natural soils, even under highly alkaline conditions, the pH value is low in the immediate neighborhood of root hairs, because of CO<sub>2</sub> production. Rapid oxidation of nitrites to nitrates even under alkaline conditions in nature is not only conceivable but logical. This also shows that the view expressed by Corbet (3) that, in the oxidation of nitrites by soils, the pH value should be within 5.8 is not entirely correct. Though it is true that at higher pH values, the rate of conversion slows down considerably, there is no doubt that it does take place. When, however, the soil acidoid is completely neutralized by treatment with

TABLE 12  
*Oxidation of KNO<sub>2</sub> by soils having pH values higher than 7*  
 Amount of KNO<sub>2</sub> added, 0.212 gm.

	pH VALUE	KNO <sub>2</sub> DECOMPOSED	
		After 30 days	After 50 days
<i>Alkali added</i>		gm.	gm.
NaOH.....	7.32	.0108	.0195
NaOH.....	8.72	0	.0151
Ca(OH) <sub>2</sub> .....	7.1	.0116	.0182
Ca(OH) <sub>2</sub> .....	8.4	0	.0141
<i>Natural soils</i>			
P.C. 13.....	8.5	.020	
P.C. 123.....	8.0	.042	
P.C. 155.....	8.9	.035	
Rawalpindi clay.....	...	.050	
Blank KNO <sub>2</sub> solution.....	8.4	0	

alkali equivalent to its total base-exchange capacity, it loses its oxidizing capacity entirely. The results obtained with two fully base-saturated soils after being shaken with nitrite solutions for 1 month showed no oxidation of nitrites. These results also offer another logical explanation of the decreased productivity of highly alkaline soils. It is obvious that excessive alkalinity might lead to the complete inhibition of nitrate formation.

As different soils have different base-exchange capacities, it is evident that the amounts of nitrites oxidized in a given time by equal weights of different soils would be different on account of their different contents of acidoid. The results would be somewhat similar to those obtained when different weights of P.C. 13 acid-treated soil or different quantities of 0.1 N HCl were taken (table 10).

To study this aspect, a number of acid-treated soil samples differing in base-exchange capacity were selected, and to 5-gm. portions of each, 25 cc. 0.1 N

TABLE 18  
*Oxidation of KNO<sub>3</sub> with different acid-treated soils*  
 0.212 gm. KNO<sub>3</sub> added to 5 gm. soil

SOIL	INITIAL pH	BASE-EXCHANGE CAPACITY <i>m.e./100 gm.</i>	KNO <sub>3</sub> OXIDIZED IN 13 HOURS
			<i>gm.</i>
13	3.5	58	.2060
32	....	44	.1870
68	4.5	5.6	.0086
70	4.2	14.0	.0285
72	....	14.0	.0106
92	4.95	12.0	.0250
107	4.4	6.0	.0086
108	4.25	12.0	.0261
110	4.9	18.0	.1899
112	5.25	14.3	.0910
114	4.32	10.0	.0260
118	4.35	12.0	.0860
119	4.35	31.0	.1989
120	4.55	10.0	.0220
121	....	12.6	.0200
122	4.45	11.0	.0241
124	4.02	14.0	.0460
126	4.7	16.0	.0893
127	4.15	14.0	.0796
128	3.95	21.0	.1116
129	5.1	19.0	.1116
133	4.5	4.7	0
135	4.75	8.0	.0200
136	4.4	5.7	.0020
139	4.8	8.0	.0154
140	4.6	7.0	.0046
142	3.7	43.0	.2060
144	4.6	10.0	.0156
146	3.1	52.0	.2100
152	4.15	13.9	.0105
153	4.65	11.0	.0266
155	....	14.0	.0616
156	....	20.0	.1010
161	3.7	34.0	.2000
162	3.9	36.0	.2000
173	4.8	22.0	.0776
177	5.32	12.0	.0023
184	4.32	20.0	.1220
188	....	12.0	0
220	....	25.0	.0915
246	4.25	25.0	.1412
262	6.1	6.0	.0120
266	5.35	18.0	.0022
277	....	18.0	.0135
289	....	7.0	0
Sand	6.96	....	0

$\text{KNO}_2$  was added. After the volume was made to 100 cc. in each case, the mixture was shaken for 13 hours and then analyzed for nitrites as usual. The results, given in table 13, show that higher base-exchange capacity of soils resulted in conversion of larger amounts of nitrites into nitrates, further supporting the view that it is the soil acidity which is mainly concerned in this reaction. The correlation coefficient between base-exchange capacity and the amount of nitrite oxidized was 0.83, which is highly significant.

Further evidence on this point was obtained by determining the oxidizing capacity of P.C. 13 acid-treated soil after being heated to different temperatures. It has been shown (16) that base-exchange capacity of soils decreases progressively at temperatures above 400°C. But up to 400°C. no change takes place. The amounts of potassium nitrite oxidized by 5 gm. of the soil heated to different temperatures are given in table 14. It is seen that up to 400°C. almost the entire amount of nitrite solution was oxidized within 13 hours. Beyond 400°C. there was a progressive decrease in the amount oxidized. These

TABLE 14  
*Oxidation of  $\text{KNO}_2$  by P.C. 13 acid-treated soil heated to different temperatures*  
 0.212 gm.  $\text{KNO}_2$  added to 5 gm. soil

TEMPERATURE °C.	$\text{KNO}_2$ OXIDIZED IN 13 HOURS gm.
29	.210
100	.206
200	.206
300	.207
400	.172
500	.042
600	.021
770	.020

observations are in conformity with the fall in base-exchange capacity of the soil above 400°C.

#### *Oxidation of oxalates by soils*

In a study of the decomposition of oxalates by soils, the senior author found two separate reactions to take place between soils and oxalates, the first involving precipitation of calcium oxalate as a result of base-exchange reaction (14), and the second involving slow oxidation of oxalates to  $\text{CO}_2$  by unsaturated H-soils (15). This oxidation reaction could not be ascribed to any specific substance in the soil, although the pH value was considered to play an important part in determining the rate of reaction.

An attempt was made at first to explain the course of this reaction in accordance with the mechanism already discussed in the case of oxidation of nitrites to nitrates, since oxidation of oxalic acid to  $\text{CO}_2$  in light had previously been observed (19). If this view is correct, then different acid-treated soils should

TABLE 15  
*Decomposition of calcium oxalates by soils*  
 50 cc. 0.1 N potassium oxalate added to 5 gm. soil

SOIL	INITIAL pH	pH AFTER 15 DAYS	0.1 N POTASSIUM	BASE-EXCHANGE CAPACITY
			OXALATE DECOMPOSED cc.	
13	3.5	6.5	47.8	58.0
32	3.5	6.25	36.0	44.0
68	4.5	7.2	31.8	5.6
92	4.95	7.42	35.9	12.0
108	4.25	7.6	25.7	12.0
110	4.9	7.3	31.0	18.0
112	5.25	7.35	27.2	14.3
114	4.32	7.4	35.6	10.0
118	4.35	7.1	46.7	12.0
119	4.35	6.72	45.6	31.0
120	4.5	7.65	23.4	10.0
122	4.45	7.1	42.1	11.0
123	4.7	7.0	33.8	25.3
124	4.02	7.3	35.3	14.0
126	4.7	7.37	44.3	16.0
128	4.2	6.85	32.2	21.0
129	4.9	7.4	29.8	19.0
133	4.55	7.1	46.3	4.7
135	4.75	7.3	35.3	8.0
136	4.4	6.95	45.1	5.7
138	4.45	7.15	20.9	7.6
139	4.52	7.12	16.2	8.0
140	4.65	7.5	25.8	7.2
144	4.6	7.1	25.5	10.0
149	4.05	6.9	46.2	18.7
153	4.8	7.38	26.6	11.0
172	4.3	6.82	47.6	29.8
173	4.8	7.02	45.2	22.0
177	5.32	6.42	30.4	12.0
184	4.32	6.84	32.4	20.0
241	5.0	7.4	29.4	18.0
242	4.7	7.2	25.6	10.0
243	4.6	6.65	36.0	15.0
244	3.8	7.25	25.0	22.0
246	4.25	7.02	43.0	25.0
247	5.2	6.65	8.6	7.0
248	6.0	7.55	23.3	8.0
249	4.3	6.95	17.6	20.2
250	5.2	7.62	25.4	25.1
255	6.0	7.62	35.1	12.4
262	6.1	7.2	44.5	14.0
263	6.88	7.25	40.0	16.0
266	5.35	7.65	20.0	6.0
184	3.2	7.0	22.4	29.9

decompose oxalates at different rates depending upon their base-exchange capacity. In order to test this point, a number of acid-treated soils were se-

TABLE 16

*Effect of neutralizing P.C. 18 acid-treated soil to different degrees on the decomposition of potassium oxalate*

50 cc. 0.1 N potassium oxalate added to 5 gm. soil

AMOUNT OF ALKALI ADDED cc.	pH OF SOIL AFTER SHAKING	POTASSIUM OXALATE DECOMPOSED cc.
<i>Neutralization with 0.1 N NaOH</i>		
10	5.36	48.7
20	5.73	48.3
30	6.44	48.3
40	7.32	47.6
50	8.72	45.8
<i>Neutralization with 0.1 N Ca(OH)<sub>2</sub></i>		
10	5.0	48.7
20	5.72	48.7
30	5.98	48.6
40	6.4	48.2
50	7.2	46.4

TABLE 17

*Decomposition of potassium oxalates by different oxides and by ferroaluminosilicates*  
50 cc. 0.1 N potassium oxalate added to 2 gm.

OXIDE	POTASSIUM OXALATE DECOMPOSED cc.	OXIDE	POTASSIUM OXALATE DECOMPOSED cc.
Al <sub>2</sub> O <sub>3</sub>	10.0	Cr <sub>2</sub> O <sub>3</sub>	2.9
Fe <sub>2</sub> O <sub>3</sub>	6.2	CuO	6.1
SiO <sub>2</sub>	12.1	PbO	1.4
BaO	6.2	MnO <sub>2</sub>	30.8
CaO	5.0	ZnO	1.1
COMPOSITION		RATIO SiO <sub>2</sub> :Fe <sub>2</sub> O <sub>3</sub> + Al <sub>2</sub> O <sub>3</sub>	POTASSIUM OXALATE DECOMPOSED AFTER SHAKING FOR 15 DAYS cc.
Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>		
per cent	per cent	per cent	
19.3	2.7	55.7	20.2
11.2	6.8	57.2	28.4
3.3	13.9	71.2	35.2
0.6	16.4	73.4	43.4

lected, and to each 5-gm. portion, 5 m.e. of potassium oxalate was added and the volume made to 100 cc. The bottles containing the mixtures were kept in

a mechanical shaker, which was worked for about 6 hours a day in diffused sunlight. After 15 days the amounts of oxalates decomposed were determined. The results, given in table 15, show that the amounts of oxalates decomposed were quite independent of the base-exchange capacities of the soils. Consequently, this reaction could not be explained on the same basis as that of oxidation of nitrites to nitrates.

Further evidence on this point was obtained by determining the amounts of potassium oxalate oxidized by P.C. 13 acid-treated soil in 10 days, after neutralization to different degrees with NaOH as well as with Ca(OH)<sub>2</sub>. The values given in table 16 show that the soil oxidized nearly the same amounts at all pH values.

Further tests made on a number of natural soils showed that, in a given time, about the same amounts of potassium oxalate were oxidized as by acid-treated soils.

From all this, it is obvious that soil acidity, as such, has nothing to do with the oxidation of oxalates. As the reaction likewise cannot be ascribed to any microbiological factor, because soils were found to retain their oxidizing property intact even when sterilized, it remained only to test the various main constituents of soil, namely, SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, and Fe<sub>2</sub>O<sub>3</sub>. To 2 gm. of each of these oxides, 5 m.e. of potassium oxalate was added, and the amounts decomposed were determined after 1 week. The results, given in table 17, show that, considerable amounts of oxalates were oxidized. Similar experiments with ferroaluminosilicates of different composition showed oxidation of oxalates to varying degrees within a week.

These results should leave no doubt that the cause of decomposition of oxalates by soils lies in the very nature of the soil composition, all the three main constituents acting as oxidizing agents. Whether these oxides act as mere catalysts in the oxidation of oxalates or themselves take part in the chemical reaction are points still under investigation.

#### CONCLUSIONS

The oxidation of nitrites to nitrates by soils takes place by a purely physico-chemical process, and quite independently of microbiological and photochemical agencies, due to the acidity of the soil.

Different soils oxidize nitrites at different rates depending upon their base-exchange capacities.

The oxidation of oxalates by soil is brought about by its components, alumina, ferric oxide, and silica.

#### REFERENCES

- (1) ASHBY, S. F. 1934 Some observations on "nitrification." *Jour. Agr. Sci.* 2: 52-67.
- (2) CORBET, A. S. 1934 Formation of hyponitrous acid as an intermediate compound in the biological or photo-chemical oxidation of ammonia to nitrous acid. *Bio-chem. Jour.* 28: 1575-1583.
- (3) CORBET, A. S. 1936 The biological and chemical oxidation of ammonia to nitric acid. *Trans. 3rd Internat. Cong. Soil Sci.* (1935) 1: 133-134.

- (4) DA ROSSI, G. 1933 Nitrification in soils by simple physico chemical action. *Bul. Soc. Internat. Microbiol. Sez. Ital.* 5: 182-186. [Abstract in *Chem. Abs.* 27: 5860.]
- (5) DHAR, N. R., BHATACHARYA, A. K., AND BISWAS, N. N. 1933 Influence of light on ammonification and nitrification in soil. *Jour. Indian Chem. Soc.* 10: 699.
- (6) DHAR, N. R., BHATACHARYA, A. K., AND BISWAS, N. N. 1933 Photonitrification in soil. *Soil Sci.* 35: 281-284.
- (7) DHAR, N. R., AND RAO, G. G. 1933 Nitrification in soil and air a photo-chemical reaction. *Ray Commemoration Vol. Jour. Indian Chem. Soc.*: 81.
- (8) DHAR, N. R., AND TANDON, S. P. 1936 Oxidation of nitrites to nitrates in sunlight. *Jour. Indian Chem. Soc.* 13: 180-184.
- (9) FRAPS, G. S., AND STERGES, A. J. 1935 Effect of sunlight on the nitrification of ammonium salts in soils. *Soil Sci.* 39: 85-94.
- (10) LYONS, T. L., AND BIZZEL, J. A. 1909 Some conditions favoring nitrification in soils. *Science* (n.s.) 30: 773-774.
- (11) McBRATH, L. G., AND SMITH, N. R. 1914 The influence of irrigation and crop production on soil nitrification. *Centbl. Bakt.* (II) 40: 24-51.
- (12) MUNTZ, G. 1928 Quoted in Mellor's comprehensive treatise on Inorganic and Theoretical Chemistry, vol. 8, Sect. on Biochemical Fixation of Nitrogen.
- (13) PURI, A. N. 1930 Studies in soil colloids: I. Base exchange and soil acidity. *Mem. Dept. Agr. India* 11 (1).
- (14) PURI, A. N. 1936 Estimating exchangeable calcium and other cations in soil. *Soil Sci.* 42: 47-59.
- (15) PURI, A. N. AND ASGHAR, A. G. Interaction between oxalates and soil. *Sir S. S. Bhatnagar Commemoration Volume, Indian Chem. Soc., Lahore Branch*: 87-94.
- (16) PURI, A. N., AND ASGHAR, A. G. 1940 Physical characteristics of soils: V. Effect of ignition. *Soil Sci.* 49: 369-378.
- (17) RAO, G., AND DHAR, N. R. 1931 Photosensitized oxidation of ammonia and ammonium salts and the problem of nitrification. *Soil Sci.* 31: 379-383.
- (18) RAO, G. 1934 Newer aspects of nitrification: I. *Soil Sci.* 38: 143-159.
- (19) RICHTER, V. 1923 Organic Chemistry, vol. 2. (Kegan Paul, Trench, Trubner & Co. Ltd.

# POSSIBLE ROLE OF BORON IN TOBACCO FERTILIZATION

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Boron is just as essential as any other plant-food element. The writer first demonstrated its essentiality to the tobacco plant in 1927 (9).

Actual boron-deficiency symptoms in tobacco have never been observed in the field in the Connecticut Valley. Frequently, however, inferior growth of tobacco has been observed in some fields where soil and plant tests have revealed nothing radically out of order in the fertility level and where pathological disease factors have been eliminated.

Crops vary in their capacity to remove from the soil the different kinds of plant food. Rotation of fast-growing crops, which are heavy feeders, with slow-growing crops of low plant-food requirements, is known to be both practical and profitable.

In the Connecticut Valley, tobacco does not respond favorably to the practice of crop rotations. When rotation is practiced, it has been found that most preceding crops, with the exception of potatoes, have an adverse effect on tobacco. When grown after corn, for instance, tobacco frequently suffers from a malady called "brown root rot."

The fact that brown root rot does not always occur in the corn-tobacco combination occasions doubt that the preceding crop in itself is the basis of the trouble. It is possible that the fertility level or the balance of nutrients in the soil has a more direct influence on production of tobacco. Because of the magnitude of the crop, corn is a greedy feeder especially on minor elements such as boron. Therefore, corn may remove from the soil constituents needed for a healthy tobacco crop.

Earlier experiments (1) showed that tobacco on previously manured land gave vastly higher return values than tobacco grown where only commercial fertilizer had been used on preceding crops. This suggested that stable manure would be suitable material to overcome adverse effects of corn preceding tobacco.

The purpose of this paper is to report on studies of growth, quality, and some of the chemical constituents of Havana Seed tobacco grown after corn and on old tobacco soils with and without the addition of boron to the soil. It also includes a study on the possible need of boron for tobacco as it is grown in the Connecticut Valley.

## METHODS

### *Field experiments*

A field was selected on which corn had been grown in 1940. After the corn-stalks were removed, one third of the field was manured at the rate of 20 tons per

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acre, another third received nitrogen, equivalent to that in manure, in the form of soybean oil meal (200 pounds of nitrogen per acre), and a third, the check plot, received no treatment. The entire field was harrowed and left without cover crop until the following spring. In May, 1941, the field was fertilized at the rate of 2,500 pounds 8-4-8 per acre. Havana Seed tobacco was planted across the treated parts.

In the fall of 1941, cornstalks amounting to the stalk yield expected for an area of equal size were harrowed into the soil of the check plot, and the entire field was sown to rye cover crop. In the spring of 1942 this field was fertilized at the rate of 2,500 pounds (8-4-8) per acre. Havana Seed tobacco was planted late in May.

Adjacent to the plots was an area planted to tobacco to fill out the field. Tobacco had been grown there for two years, and corn previously to that. In both years the tobacco appeared inferior to that of the test field. A part of this area, and the experimental plots as well, were harvested for data.

In order to observe the effect of boron and of boron in combination with lime on quality and yield of tobacco, a field was chosen that had grown corn in 1942. The soil was Merrimac coarse sandy loam with a pH of 5.0 and of normal fertility. A strip across the center of the field was limed at the rate of 1,000 pounds of calcic hydrated lime to the acre. The entire field was fertilized at the rate of 2,500 pounds of 8-4-8 to the acre. In lining up the field for planting tobacco, rows were laid out crosswise to the limed strip.

Borax was applied on both sides of the row mark at rates of 10 and 20 pounds to the acre, a sufficient number of rows being left untreated as checks. The test was made in duplicate, with the replicates in opposite parts of the field, which was then planted to Havana Seed tobacco.

In order to observe further the effect of borax applications to tobacco land, another experiment was made in 1944. The land selected had grown tobacco previously for 4 years, averaging about 1,950 pounds tobacco per acre with a grade index of 0.430 for the 4-year period.

The soil is a fine sandy loam of the Merrimac series. Twelve  $\frac{1}{4}$ -acre plots were laid out in a randomized arrangement, of which four plots were checks, four received the equivalent of 10 pounds of borax per acre, and the remaining four, 20 pounds.

After the land was fertilized (2,500 pounds 8-4-8 per acre) and fitted the row were marked off. Borax was applied partly *in the mark* for the first two replicates of both rates, and it was applied on *both sides of the mark* for the other replicates. The field was then planted with Havana Seed tobacco.

#### *Chemical investigations*

Calcium and potassium in plant materials were determined according to standard methods. Replaceable calcium in soils was determined on a weighed amount of soil, with known moisture content. The sample was shaken with universal soil-leaching solution (5) for half an hour and calcium was determined on an aliquot, according to standard methods.

Boron and phosphorus in plant materials were determined spectroscopically.<sup>2</sup> Boron in soils was determined by a method based on Morgan's micro-method (5). A weighed sample of air-dried soil was shaken with a quantity of extracting solution for half an hour. An aliquot was taken down to dryness and treated with the reagents used in proportions as described by Morgan. For obtaining the desired volume, a quantity of 95 per cent ethyl alcohol (washed) was employed. Colors were compared in a Fisher's photolometer. The results checked with the Berger-Truog method.<sup>3</sup> The same method was used to estimate boron in the ash of fertilizer materials.

Nitrate nitrogen in tobacco was determined by a modified Emmert (4) method. The modification consisted in using a Waring Blender to obtain the extract (3 minutes for each sample). Colors, obtained by phenoldisulfonic acid, were compared in a Fisher's photolometer. Aluminum was estimated in the ash from samples of tobacco by the logwood method (2). Colors were compared in a photolometer.

TABLE 1  
*Yield and grading results from corn-tobacco experiment—1941 crop*

TREATMENT	YIELD PER ACRE	GRADE INDEX	RELATIVE CROP VALUE
			lbs.
Check.....	2,445	.420	100
Manure.....	2,295	.445	100
Soybean meal.....	2,535	.418	103

#### EXPERIMENTAL RESULTS

Results on yield and grading of tobacco from the 1941 test are given in table 1. They suggest no adverse effect on tobacco grown on land previously cropped to corn. The fertility levels, as revealed by microchemical tests, were virtually the same on all three lots, with soil reactions of about pH 6.0.<sup>4</sup>

Table 2 includes yield and grading records for the three plots in the 1942 experiment and also for that in the filled-out section of the field (identified as "poor growth"). The land to which cornstalks were added produced both higher yield and grading than did the plots treated with manure and soybean oil meal in 1941. The "poor growth" area produced only "stemming" and, with respect to quantity, only half yield.

This limited experiment suggests that the growing of corn previous to tobacco

<sup>2</sup> For those determinations, the author is indebted to A. T. Myers and J. L. Showacre, of the Bureau of Plant Industry Station, Department of Agriculture, Beltsville, Maryland.

<sup>3</sup> The writer is indebted to Evelyn Smith, of the soils department, Connecticut Agricultural Experiment Station, New Haven, for checking the boron data by the Berger-Truog method.

<sup>4</sup> Connecticut tobacco usually is not grown on land with a soil reaction as high as pH 6.0, since black root rot often occurs at this level. The strain employed was resistant to this disease, however, and no root rot was observed.

*per se* had no adverse effect on tobacco. In other words, the cornstalk itself did no harm in rotation with tobacco.

Soil tests made after the removal of the tobacco crop showed that the fertility status of the corn (check) plot was on the same level as the other two lots, in respect to main nutrients and minor elements. pH values varied from 5.5 to 5.8. The "poor growth" area showed a pH value of 5.3 and a lower level of phosphorus, potash, and calcium, but not lower than is commonly found in many tobacco fields of satisfactory production. It is possible that improper balance of nutrients in the soil was responsible for the vast differences in the two areas, that is, the "poor growth" area versus the three plots.

#### *Balance of soil nutrients*

On the theory that improper balance of nutrients in the soil was responsible for the aforementioned differences, the literature in the field was reviewed prior to more detailed analysis of the data.

In the course of experimental work at this station, the proper amounts of nitrogen, phosphorus, and potash required for a cigar leaf tobacco crop have been well established. In addition, calcium and magnesium have received attention with respect to suitable amounts to apply and their effects on quality of tobacco. Minor elements, such as manganese, also have been studied in regard to plant requirements and quality. The requirement of boron for tobacco has been established, although the effect of this element on yield and quality has received but little attention.

There are many combinations of nutrients in which the magnitude of any one element might influence the magnitude of another, as may be judged by the final deposition in the tobacco leaf. Potassium and calcium are two of the most abundant elements found in the tobacco leaf, and it was thought that the balance between the two might have had an influence on the differences in crop values as reported in table 2.

Representative samples of tobacco from "darks" and "seconds" were analyzed with respect to potassium and calcium. The results are listed in table 3. To be sure, the analytical data show no striking differences between the treatments. The balance between potassium and calcium, as expressed by the K/Ca ratio, suggests no trend whatever. There is, however, a tendency for the calcium content to increase as yield and quality of tobacco improved (compare table 2). This is in line with previous work on calcium nutrition, as reported by the writer (11) in 1941.

Calcium alone could not possibly have been responsible for the vast differences encountered, especially between the crop values from the "poor growth" area and that of the cornstalk-treated lot.

A close association is known to exist between calcium and boron with respect to absorption and utilization of calcium by plants. This was first pointed out by Warington (12) in 1934. Her views were supported by Swanback (10) in 1939 and more recently by others. Drake (3) and co-workers have asserted that a certain ratio between calcium and boron would be most suitable for proper development of tobacco. By means of plant analyses they found that ratios below

1,500:1 produced normal growth, whereas wider ratios caused boron-deficiency symptoms.

The value of a tobacco crop ("stalk cut") is largely determined by its content of more mature leaves: seconds, light wrappers, and mediums. In these types of leaves, potassium, calcium, and boron are stored in the cells. It is a commonly accepted fact that, of the three elements, only potassium can be readily translocated to the upper parts of the plant, whereas calcium and boron must be steadily supplied from the culture media. Calcium and boron, therefore, should be readily available in the soil at all times during the growing season.

TABLE 2  
*Yield and grading records from corn-tobacco plots—1942 crop*

TREATMENT	YIELD PER ACRE	GRADE INDEX	RELATIVE CROP VALUE
	lbs.		
"Poor growth".....	875	.190	22.5
Manured 1941.....	1,643	.381	84.6
Soybean meal 1941.....	1,655	.415	92.8
Check, cornstalks 1942.....	1,770	.418	100.0

TABLE 3  
*Potassium and calcium content of tobacco from corn-tobacco plots in 1942*

TREATMENT	GRADE	K	Ca	AVERAGE RATIO K/Ca
"Poor growth"	Darks	per cent	per cent	.884
	Seconds	3.73	3.44	
	Ave.	3.45	4.68	
Manured 1941	Darks	3.59	4.06	.942
	Seconds	3.97	4.04	
	Ave.	4.17	4.59	
Soybean meal 1941	Darks	4.07	4.32	.844
	Seconds	3.81	4.14	
	Ave.	3.67	4.71	
Corn + stalks 1942	Darks	3.74	4.43	.866
	Seconds	3.82	4.37	
	Ave.	4.00	4.65	

Yield and grading data of the 1943 crop (table 4) indicate that the relative crop values, as listed below, were not affected by liming in itself:

Check, no lime.....	100.0
10 lbs. borax per acre, no lime.....	105.3
20 lbs. borax per acre, no lime.....	112.1
Check, limed.....	102.4
10 lbs. borax per acre, limed.....	100.0
20 lbs. borax per acre, limed.....	114.1

Ten pounds of borax, *without lime*, resulted in a somewhat higher value than the check. Twenty pounds of borax to the acre, however, definitely improved the crop values both on limed and unlimed parts of the field. The results indicate that this particular field had a satisfactory calcium content without liming but produced more and somewhat better tobacco when sufficient boron was added to the soil.

Moreover, the tobacco from the check plots must be considered quite satis-

TABLE 4  
*Yield and grading records of boron plots, 1943 crop*

TREATMENT		PLOT	YIELD PER ACRE	PERCENTAGES OF GRADES*							GRADE INDEX
Borax lbs.	Lime			L	M	LS	SS	LD	DS	F	
0	No	A	2,250	8	6	36	6	38	0	6	0 .470
		B	2,438	4	4	45	4	26	0	4	.441
		Ave.	2,344								.456
0	Yes	A	2,179	6	6	30	12	42	0	4	0 .442
		B	2,384	11	10	39	3	34	0	3	.518
		Ave.	2,281								.480
10	No	A	2,326	8	7	32	4	44	0	5	0 .463
		B	2,344	10	7	40	6	32	0	5	.501
		Ave.	2,335								.482
20	No	A	2,557	13	7	30	9	33	2	6	0 .488
		B	2,596	1	2	46	4	42	0	5	.441
		Ave.	2,577								.465
10	Yes	A	2,415	7	6	36	10	35	0	6	0 .463
		B	2,182	9	2	36	6	42	1	4	0 .468
		Ave.	2,299								.465
20	Yes	A	2,242	21	10	21	7	34	0	7	0 .526
		B	2,530	10	4	40	3	40	0	3	0 .496
		Ave.	2,386								.511

\* L = lights; M = mediums; LS = long seconds; SS = short seconds; LD = long darks; DS = dark stemmings; F = fillers; B = brakes.

factory both in yield and grading. Thus, again, tobacco was successfully grown after corn. In this case also, the corn field possessed a fertility level on a par with that of the tobacco land, as indicated by microchemical soil tests.

It is suggested from the findings of Reeve and Shive (6), that corn, because of the size of the crop, is a relatively heavy feeder on boron. This crop, therefore, may reduce the boron content of the soil to such a degree that succeeding crop yields may be reduced or totally fail. This might explain why tobacco sometimes fails after corn.

The favorable results from borax applications led to further studies on nutrient absorption by tobacco. Samples of tobacco leaves from this experiment were analyzed for potassium, calcium, and boron. The results are given in table 5.

Tobacco from borax-treated plots had not only a higher boron content, increasing with higher soil treatments, but also a higher potassium content (25 per cent increase). Calcium was slightly decreased. The behavior of these elements was in line with previous work by the writer, who found that, in the presence of boron, potassium increased in the plant, irrespective of the magnitude of potassium and calcium in the culture media (10, Table IX), as compared with boron-free cultures; calcium was only slightly affected.

TABLE 5  
*Content of calcium, potassium, and boron in tobacco leaves from boron test, 1943 crop*  
 Results on air-dry basis

TREATMENT		PLOT	CALCIUM			POTASSIUM			BORON		
Borax	Lime		Seconds	Darks	Ave.	Seconds	Darks	Ave.	Seconds	Darks	Ave.
lbs./A.			per cent	per cent	per cent	per cent	per cent	per cent	p.p.m.	p.p.m.	p.p.m.
0	No	A B	3.72	2.88	3.08	3.28	2.49	2.92	33	36	49
			2.94	2.76		3.18	2.71		52	74	
0	Yes	A B	4.32	3.96	3.82	2.71	3.14	3.16	35	34	42
			3.60	3.59		3.25	3.55		50	49	
10	No	A B	3.00	2.40	2.79	3.69	4.18	3.87	80	81	78
			3.59	2.40		3.94	3.69		78	71	
20	No	A B	3.59	2.52	3.09	3.02	3.40	3.97	152	113	120
			3.60	2.88		4.06	5.39		118	96	
10	Yes	A B	3.72	2.85	3.15	3.50	3.75	3.36	85	69	80
			3.59	2.64		2.71	3.49		89	79	
20	Yes	A B	4.08	3.12	3.48	3.74	4.08	3.95	122	87	99
			3.84	2.88		3.83	4.17		98	90	

The balance of nutrient elements is clearly visualized when expressed as ratios (table 6). The potassium-calcium ratios, where no borax was added, are almost identical to those found in table 3. Addition of borax, however, greatly increased the values. When the lime (calcium) factor is omitted, the ratios for the two rates of borax applications are almost identical. In this comparison, potash is more than one third higher in tobacco from the borax-treated plots than in that from the check plots, irrespective of liming.

The calcium-boron balance, referred to earlier in this article, is in good agreement with the work by Drake and co-workers (3). Even in tobacco from the check plots the ratios are well below the critical limit of 1,500:1, and it must be recalled that the returns from these plots were entirely normal. It is indicated,

however, that the calcium-boron ratios must be narrowed considerably in order to bring about an improvement in already rather satisfactory crop values. Applications of 10 pounds of borax per acre narrowed the ratio from 770:1 to 376:1 without significantly affecting the relative crop values (100 and 102). With 20 pounds of borax to the acre, however, a still narrower ratio was obtained, 305:1, effecting a relative crop value of 112.

It is conceivable that a single ratio might express the interrelation of potassium, calcium, and boron. In such a ratio the product of potassium and boron (based on contents of the crop) is weighted against calcium. The "triple element" ratios (table 6) show, in general, a progressive widening with improvement in relative crop values. These calculations suggest that ratios approaching 100:1 are correlated with high crop values and that wider ratios are correlated with higher relative crop values.

TABLE 6  
*Average ratios, based on data in table 5 and relative crop values, 1943 test*

TREATMENT		K/Ca	Ca/B	(K X B)/Ca	RELATIVE CROP VALUE
Borax lbs./A.	Lime				
0	No	0.95	629	46	100
	Yes	0.83	910	35	102
10	No	1.39	358	108	105
20	No	1.28	258	154	112
10	Yes	1.07	394	85	100
20	Yes	1.14	352	112	114
0		0.89	770	41	100
10		1.23	376	97	102
20		1.21	305	133	112

#### *Borax applications on old tobacco land*

Where 20 pounds of borax per acre had been applied partly *in the mark*, a great number of the plants died, and some of the plants died even after restocking. All through the growing season the tobacco on these plots lacked the vigor shown on the remainder of the field.

Despite an unusually dry season, the yield and grading results (table 7) must be considered satisfactory. Higher yields and grading resulted from 10 pounds borax per acre, than from the checks and the 20-pound rate. If replicates A and B, where results were inferior (probably because of borax injury), were omitted, the 20-pound rate would have a better showing.

The average relative crop value for the 10-pound borax application is almost identical with the value obtained in previous experiment with 20 pounds borax per acre. This could mean that the soil in the latter case originally contained

less boron than the soil of the 1944 test. Soil investigations were therefore undertaken.

### *Soil investigations*

After removal of the 1944 crop, soil samples were collected from the 12 plots. Preliminary microchemical tests (Morgan's universal soil-testing system) revealed a very high content of nitrate nitrogen, more than ample amounts of phos-

TABLE 7  
*Yield and grading records of tobacco in borax-application tests, 1944 crop*

BORAX APPLICATION PER ACRE	PLOT	YIELD PER ACRE	PERCENTAGES OF GRADES*						GRADE INDEX	
			L	M	LS	SS	LD	DS		
0	A	1,838	1	2	33	9	38	8	9	.386
	B	2,127	1	1	41	5	40	4	8	.413
	C	2,191	1	4	25	12	44	7	7	.373
	D	2,029	1	1	24	13	43	11	7	.357
	Ave.	2,046								.382
10	A	2,036	6	7	27	10	40	4	6	.428
	B	2,191	7	4	26	12	39	7	5	.422
	C	2,112	7	7	25	10	37	8	6	.425
	D	2,114	1	2	33	9	42	8	5	.394
	Ave.	2,113								.417
20	A	1,697	..	..	32	7	43	10	8	.370
	B	1,864	2	2	25	14	36	13	8	.376
	C	2,037	6	6	28	10	37	7	6	.425
	D	2,108	1	2	30	9	43	10	5	.383
	Ave.	1,926								.389

\* See footnote to table 4.

### Summary:

Borax Applications per Acre lbs.	Average Crop Index	Relative Crop Value
0	781.57	100.
10	881.12	112.7
20	749.21	95.9
[20]	[837.09]	[105.8]

The data in brackets represent replicates C and D.

phoric acid, very high potash content, medium to medium high content of calcium, and high content of magnesium. Soil reactions varied from pH 5.20 to 5.65, with most of the tests bordering pH 5.33. The relatively high content of nitrate nitrogen can be accounted for by the extremely dry season, resulting in no leaching and insufficient moisture for an excessive absorption of nitrogen by the plants. In table 8 the replaceable calcium and boron found in the soils of the 12 plots are reported.

The calcium content of these soils must be considered adequate for proper nutrition of tobacco, according to previous work by the writer (11).

With one exception, the boron content of the soil from the check plots was about 0.5 p.p.m. More than three times this amount was present in the soil receiving borax. It must be recalled, however, that the borax was applied as a side-dressing. This strip therefore, received about three times as much as the land would have received had the material been distributed broadcast over the entire area of the plots.

Theoretically, about 4 p.p.m. of boron would have been present at the start in the soil strip, where the plants grew, on plots receiving 10 pounds of borax to the acre, and almost twice this amount at the 20-pound rate. Since no injury to the plants occurred at either rate where borax was applied on each side of the rows of

TABLE 8  
*Replaceable calcium and boron in soils from borax test of 1944*

BORAX APPLICATION PER ACRE	PLOT	CALCIUM p.p.m.	BORON p.p.m.	Ca/B
lbs.				
0	A	740	0.48	1,542
	B	820	0.53	1,547
	C	820	0.49	1,673
	D	975	1.08	903
	Ave.	839	0.65	1,416
10	A	775	1.40	554
	B	875	1.66	527
	C	1,040	2.30	452
	D	1,000	1.95	513
	Ave.	923	1.83	512
20	A	975	1.95	500
	B	840	1.43	587
	C	890	2.00	445
	D	1,020	1.25	816
	Ave.	931	1.66	587

tobacco, it is suggested that the tobacco plant (on this fairly typical valley tobacco land) can tolerate more than 4 p.p.m. of boron, but probably less than 8 p.p.m.

The calcium-boron balance in the soil, expressed as average ratios in table 8, is somewhat reflected in the relative crop values (table 7). Thus better returns can be expected with a relatively narrow ratio in the soil.

#### *Fertilizer practices and boron content of tobacco soils*

It is commonly believed that tobacco fertilizers, because of their high content of organics, can easily maintain the boron content of the soil. Reeve and co-workers (7) have asserted that organic materials are not adequate sources of boron, but none of the fertilizer ingredients most commonly used in mixtures for

tobacco, except cottonseed meal, was included in their data. The boron content of these materials was therefore determined, as follows:

	p.p.m. B
Cottonseed meal.....	16.5
Castor pomace.....	24.0
Soybean oil meal.....	21.0
Bone meal (steamed).....	22.7
Cottonhull ashes.....	28.5
Landplaster (gypsum).....	28.5
Sulfate of potash (imported).....	29.4
Triple superphosphate.....	25.5
Nitrate of soda (synthetic).....	Trace
8-4-8 tobacco fertilizer mixture.....	19.5

TABLE 9  
*Nitrate-nitrogen content of tobacco leaves of boron test, 1943 Crop*

TREATMENTS		PLOT	NO <sub>3</sub> - N			NO <sub>3</sub> - N X B*	RELATIVE RATIO VALUES
			Seconds	Darks	Ave.		
Borax	Lime		per cent	per cent			
lbs./A.		A	.0233	.0181	.0188	9,212	100
			.0171	.0176			
0	Yes	A	.0151	.0168	.0141	5,880	64
			.0111	.0130			
10	No	A	.0266	.0289	.0237	18,486	200
			.0191	.0201			
20	No	A	.0145	.0246	.0206	24,720	268
			.0205	.0228			
10	Yes	A	.0211	.0314	.0233	18,640	202
			.0201	.0205			
20	Yes	A	.0119	.0181	.0183	18,117	200
			.0240	.0191			

\* Considered as whole numbers instead of decimal fractions.

On the basis of these data, 3,000 pounds of cottonseed meal (the maximum usually applied per acre) would furnish less than  $\frac{1}{2}$  pound of borax per acre. Considerably smaller quantities of the other organics are used.

The 8-4-8 mixture would furnish the equivalent of  $\frac{1}{3}$  pound of borax per ton, and the regular rate of application per acre (2,500 to 2,800 pounds) would be equivalent to about  $\frac{1}{2}$  pound of borax.<sup>5</sup> Thus the materials added in commercial

<sup>5</sup> As a check on the estimation of boron in the materials listed, a theoretical 8-4-8 was composed. This contained 0.0385 pound B whereas the actual material analyzed 0.039 pound B per ton.

fertilizers would *not* supply sufficient boron for replenishing this element in the soil.

The 200 pounds of nitrogen in an average application, however, may effect a liberation of boron from the soil minerals by virtue of the nitrification processes. According to Reeve and co-workers (7) organic acids increase the water-soluble boron in the soil. Undoubtedly, the mineral acids formed in the soil would have an even greater dissolving action on boron-containing minerals.

Scripture and McHargue (8) and more recently, White-Stevens and Wessels (13) found an interaction between nitrogen and boron in spinach and rutabagas, respectively. The latter investigators concluded that fertilizer nitrogen (nitrate of soda and ammonium nitrate) reduces boron-deficiency symptoms.

The following observations were made in comparisons of the nitrate-nitrogen (table 9) and boron contents of the 1943 crop (table 5):

In general, the nitrate-nitrogen content is higher where the crop received borax.

Addition of lime to the soil (without borax) decreased the nitrate content.

If the interaction of nitrate and boron is expressed as a product of the two constituents, there is a tendency toward stability with applied borax. This stability is also present in the seconds (lower part of the plant) versus the darks (upper part of plant), as may be seen from the following: The average content of nitrate nitrogen of all seconds (irrespective of treatments) is found to be 0.0186 per cent; of all darks 0.0209 per cent (a difference of about 13 per cent). The average content of boron (from table 5) for corresponding grades is 83 and 73 p.p.m., respectively (a difference of little over 13 per cent, in an opposite direction, however). The products, calculated as above, show a remarkable resemblance in magnitude, 15,438 and 15,257, for seconds and darks, respectively. This may be considered as another example of the varied effect of boron in plant life.

Results from phosphorus determinations on the 1943 crop showed no correlation with borax applications or additions of lime. The weighted average for the crop amounted to 0.24 per cent phosphorus, with a mean deviation of  $\pm .05$  per cent.

Potash, of which there is a great abundance in most tobacco land of the Connecticut Valley, must be considered a factor in maintaining a level of available boron in the soil.

From the known fact that potash and boron (6) increase together in the plant, it may be inferred that some of the boron compounds in the soil may be present as borates of potassium. These have a much higher solubility than boron combined with calcium, which is the dominating element in most agricultural soils. In fact, the results obtained by Reeve and co-workers (6) might very well be explained on the theory that potassium borates are formed when the influence of calcium is eliminated.

It is obvious, however, that aluminum and other amphoteric elements (Mn and Fe) in the soil have an influence at low and at relatively low calcium supply. Thus, an abundance of active aluminum in the soil would tend to cause boron to revert to the mineral status, such as boroaluminum silicates, which are very slowly available (6).

To see whether the aluminum content of tobacco might show some relation to

that of boron, the 1943 crop was examined for aluminum. The content of this element, however, showed no marked relation to either lime or borax applications, although it was noticed that the average content of the "seconds" [0.075  $\pm$  .015 per cent ( $Al_2O_3$ )] was higher than that of the "darks" [0.06  $\pm$  .014 per cent ( $Al_2O_3$ )]. The fact that the boron content went in the same direction (83 and 73 p.p.m. B) suggests that boron might enter the plant in combination with aluminum, or it may only prove the analogy in behavior known to exist between the two elements.

#### SUMMARY AND CONCLUSIONS

Studies were made from 1941 through 1944 of growth, quality, and some of the chemical constituents of Havana Seed tobacco grown in the Connecticut Valley under various fertilizer treatments on selected fields. The results lead to the following conclusions:

Brown root rot in tobacco may be due to "crop fatigue" caused by insufficient boron or improper balance of potassium, calcium, and boron in the soil.

Tobacco can be grown successfully after corn, provided the level of soil fertility of corn land is on a par with that of tobacco land in good production, including sufficient boron.

Suitable amounts of borax applied to corn land and tobacco land improved crop values as much as 12 per cent.

Potassium-calcium ratios, based on crop analyses, are widened by applied borax, that is, potassium increases in the plant while calcium remains fairly constant.

A sufficient amount of boron added to limed land will maintain a proper potassium-calcium balance.

Satisfactory yields are obtained when the calcium-boron ratio in the crop is about 900 to 1. For considerable improvement in an already satisfactory crop, the ratio must be narrowed to about 300 to 1.

Because of the close interrelation of potassium-calcium-boron, a ratio expressed by the fraction ( $K \times B/Ca$ ) is suggested. This ratio widens as the crop values increase.

On typical Connecticut Valley tobacco soil the plants can tolerate more than 4 p.p.m. of boron but less than 8 p.p.m. It would be safer not to exceed the lower figure.

The ratio of replaceable calcium to boron in soils is reflected to some degree in the corresponding ratio of the crop.

The common practice of fertilization of tobacco in the Connecticut Valley maintains the boron status in the soil, partly by small additions of boron in the fertilizers and partly through the reactions on soil constituents on the materials added.

Recommendations on a general use of borax on tobacco land must await further investigation.

#### REFERENCES

- (1) ANDERSON, P. J., AND SWANBACK, T. R. 1942 Residual effect of stable manure. *Conn. Agr. Exp. Sta. Bul.* 457: 242-243.
- (2) COLWELL, J. K., AND PARKER, A. E. 1930 The logwood test for aluminum. *Brit. Food Jour.* 2: 346-347.
- (3) DRAKE, M., SIELING, D. H., AND SCARSETH, G. D. 1941 Calcium-boron ratio as an important factor in controlling the boron starvation of plants. *Jour. Amer. Soc. Agron.* 33: 454-462.
- (4) EMMEET, E. M. 1932 Field method for estimation of nitrate, phosphate, and potassium in plants. *Plant Physiol.* 7: 315-321.
- (5) MORGAN, M. F. 1941 Chemical soil diagnosis by the universal soil testing system. *Conn. Agr. Exp. Sta. Bul.* 450.



# SEASONAL VARIABILITY IN THE AGGREGATION OF HAGERSTOWN SILT LOAM<sup>1</sup>

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The dynamic nature of soil structure has long been recognized. Since soil structure is influenced by the biological activity, the growing plant, organic and inorganic additions, the moisture regime, and the mechanical manipulations of the soil by tillage operations, it is only logical that as these vary, their effects will be manifest in the variation of the structure or the aggregation of the soil.

Several investigations have been reported in which attempts were made to study the effect of certain soil treatments on the structure of the soil at certain periods of the year. Nekrassov (14) and Apsits (4), in studying the effects of tillage operations on soil structure, measured the changes in capillary porosity throughout the growing or "vegetation" period. Andrianov (3) measured the changes in the vertical size of the plowed layer in his study of seasonal variations in the structure of the surface soil. Henin (9), who studied the effect of moisture content on the stability of soil aggregates, noted the seasonal variation in aggregate size distribution which, after 7 years of measurement, he considered to be cyclic in character. He found (10) that under the conditions at Versailles, France, water-stability of soil aggregates was at a maximum during the dry period of late summer and at a minimum in the wet period of each spring. Cole (7) estimated the effects of tillage and irrigation on the variation in soil macrostructure by sifting the air-dry field sample and then plotting the aggregate size distribution curve, whereas Kolodny and Neal (13) used the microaggregation or dispersion measurements for following the changes in soil structure.

The results of each of these investigations demonstrated that soil structure is not a static quality, but is subject to constant fluctuation.

The purpose of this investigation was to measure the effects of seasonal conditions on the aggregation of Hagerstown silt loam surface soil, upon analysis in the field-moist and air-dry conditions.

## PLOT DESCRIPTION AND TREATMENT

These investigations are based on 1/500-acre plots situated on Hagerstown silt loam soil at the Pennsylvania Agricultural Experiment Station.

Barnyard manure, wheat straw, chopped corn stover, oak leaves, and pine needles were applied in 1939 as surface mulches to some of the plots at a rate of 5 tons per acre on a dry-weight basis. The same rate of application was used in comparable plots where barnyard manure, wheat straw, and chopped corn stover

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were incorporated in the surface soil. Each succeeding year, 1940-1944, additional applications of the materials were made at half the original rate.

Sand and gravel was used as a surface mulch also at the rate of 20 tons per acre. The mulched plots remained untilled throughout the 5-year period.

Several additional treatments were used separately on another group of plots. Ryegrass was sown at the rate of 20 pounds per acre in the late summer of each year where it served as a fall, winter, and spring cover crop, and then was plowed down in the early summer when it became a source of green manure to the soil. A 4-12-8 fertilizer was broadcast at the rate of 800 pounds per acre and plowed down in the early summer of each year. Two check treatments were included for comparative purposes. In one the soil was plowed and cultivated, and in the other the soil was not disturbed by plowing or cultivation, the weeds being removed by pulling or scraping. In neither of these plots was any supplementary material added to the soil.

Field corn was planted in eight 3- by 3-foot hills on every plot in the experiment in 1943 and 1944. Each hill contained three stalks of corn.

The various materials were incorporated in the surface soil by plowing in either the late spring or early summer of each year. The tilled plots were then cultivated at three subsequent dates throughout the summer.

Since the purpose of this investigation was to study the seasonal effects on soil structure under a given set of field conditions, the effect of different cultural treatments was secondary. One plot from each of the randomized treatments was selected in which the soil was found by previous examination and analysis to be most uniform with respect to each of the other plots. The soil in all of the plots except those treated with the corn stover, ryegrass, and complete fertilizer had an organic matter content of 2.9-3.0 per cent and a field moisture capacity of 27 per cent prior to being differentially treated in 1939, whereas the latter three plots had 2.6 per cent organic matter and a field moisture capacity of 23-24 per cent. The wilting range for this soil is 10-12 per cent. The soil in each plot had a pH of 6.4-6.6. Studies of Merkle (15) on similarly treated soil of this type in the Jordan fertility plots show that in this pH range the base-exchange complex would be approximately 70 per cent saturated. Of the total milliequivalents of exchangeable bases, the relative proportion of the different cations saturating the complex is as follows: calcium 80 per cent, magnesium 12 per cent, potassium 6.5 per cent, and ammonium and sodium 1.5 per cent. The chemical and mineralogical characteristics of this soil have been presented by Jeffries and White (11). The soil in each plot contained 18 to 20 per cent clay and inorganic colloidal material. The mineralogical character of the clay in the Hagerstown soil has been identified by Jeffries<sup>2</sup> as of the kaolinitic type.

#### EXPERIMENTAL PROCEDURE

##### *Soil sampling procedure*

Soil samples were taken from each plot for aggregate analysis and moisture determination beginning in March 1944 and continued during each month except

<sup>2</sup>Jeffries, C. D. Unpublished data. Pennsylvania Agricultural Experiment Station. 1945.

January until March 1945. The surface soil in each plot was sampled at the 0-1-, 1-3-, and 3-6-inch depths. A large spoon was used in carefully removing the soil from each layer so as to minimize the disturbance in aggregation due to sampling. The results of each of the soil measurements presented represent the mean of three separate samples from each plot.

#### *Analytical procedure*

Aggregation was used as the index of soil structure. The wet-sieving principle of aggregate analysis as originated by Tuilin (20) was used in obtaining the percentage of aggregates  $>0.25$  mm. The aggregate analysis technique of Alderfer and Merkle (1) was used in determining the complete primary and secondary particle distribution. This method involved immersing the air-dry or field-moist sample in an excess of water and allowing it to slake for 24 hours. The sample was then transferred to a 1-liter graduate cylinder. The soil was agitated in water suspension by tilting the cylinder end over end ten times. A 25-cc. aliquot was then withdrawn with a pipette at the appropriate time and depth as determined by the application of Stokes' law. Following the withdrawal of the fine sand, silt, and clay aliquots, the coarser sand and gravel-sized particle fractions were separated by wet-sieving. Each sample was analyzed in this manner. That percentage of the soil which consisted of aggregates and primary particles  $>0.25$  mm. in diameter was calculated as the single-value estimate of the existing structural condition of the soil. This value is referred to in this paper as the measured amount of aggregation that exists in any given soil sample.

To determine the effect of variability of the primary particle content of the  $>0.25$  mm. aggregated fraction, a mechanical analysis of several hundred of these aggregate analysis fractions was made. The standard deviation of the primary particle content of the  $>0.25$  mm. aggregate analysis fraction was 1.8 from a mean of 14.5 gm. of primary particles  $>0.25$  mm. in 100 gm. of soil. Thus, a difference of 1.8 per cent in the aggregation data due to variability of the primary particle content of the  $>0.25$ -mm. fraction would not significantly affect the reliability of the results due to seasonal influences on aggregation.

Following each monthly sampling, an aggregate analysis was made of the soil from the variously treated plots at its actual field-moisture content without previous air-drying. To study the effect of air-drying, a portion of the soil sample from a series of treatments was air-dried to a moisture content of 1.5 per cent before subjection to an aggregate analysis.

The soil moisture content was determined by oven-drying a portion of each quartered field sample at 105°C., and the percentage soil moisture was expressed on the oven-dry weight basis.

In obtaining the aggregate analysis data for the whole soil in tables 1 and 5, a 70-mesh sieve having openings 0.2 mm. in diameter was added to the nest of 10-, 20-, and 60-mesh sieves in the wet-sieving. The size group separations consisting of the fractions  $>2.0$ , 2.0-1.0, 1-0.2, 0.2-0.02, 0.02-0.002, and  $>0.002$  mm. are referred to as the gravel, coarse sand, medium sand, fine sand, silt, and clay fractions, respectively, in the discussion of experimental results.

## EXPERIMENTAL RESULTS

*Effect of air-drying on aggregate size distribution*

In this investigation the soil under six different treatments at the April 17 sampling was analyzed at its field moisture content and after air-drying in order

TABLE 1  
*Effect of air-drying on aggregate size distribution in Hagerstown silt loam*  
Sampled April 17, 1944

SOIL TREATMENT	DEPTH inches	SOIL AGGREGATE AND PRIMARY PARTICLE SIZE CLASSES									
		> 2.0 mm.		2.0-1.0 mm.		1.0-0.2 mm.		0.2-0.02 mm.		0.02-0.002 mm.	
		Field-moist	Air-dry	Field-moist	Air-dry	Field-moist	Air-dry	Field-moist	Air-dry	Field-moist	Air-dry
Check—un-tilled	0-1	9.7*	7.5†	3.5	3.2	16.7	11.8	36.2	58.5	31.1	18.5
	1-3	12.6	7.6	10.3	3.9	26.3	17.5	40.0	63.1	10.0	7.6
	3-6	16.5	7.8	7.9	3.5	19.1	15.8	44.2	64.1	11.2	8.6
Check—tilled	0-1	10.1	5.9	2.6	3.8	12.8	12.7	38.0	59.4	36.2	17.7
	1-3	9.8	9.3	5.8	3.6	25.7	19.5	42.6	58.7	14.8	8.7
	3-6	8.8	11.5	5.2	3.8	24.9	15.0	47.7	62.3	12.1	7.3
Manure mulch	0-1	20.4	8.2	20.9	14.9	24.4	50.6	29.0	24.0	5.0	3.2
	1-3	17.7	7.9	12.8	10.5	14.9	35.2	45.0	42.5	8.9	3.8
	3-6	18.1	10.1	7.9	3.4	14.4	26.7	45.4	55.8	12.2	3.9
Manure incor-porated	0-1	12.2	8.6	4.3	3.6	18.4	17.0	41.2	54.1	22.3	16.4
	1-3	10.2	9.9	9.8	4.9	29.5	27.9	35.8	52.7	14.0	4.5
	3-6	18.2	11.1	10.7	5.0	20.4	31.8	36.4	47.6	13.4	4.4
Ryegrass cover and green manure crop	0-1	11.8	6.2	8.0	4.1	16.5	23.9	41.8	51.6	20.2	13.9
	1-3	15.2	8.8	14.7	6.1	27.4	32.3	31.8	47.5	10.4	5.2
	3-6	23.2	8.6	10.9	5.6	18.9	29.7	36.2	49.4	10.1	6.6
800 pounds of 4-12-8 fertilizer broadcast and plowed down	0-1	9.9	9.9	3.7	3.6	21.1	11.7	34.2	56.5	28.4	17.4
	1-3	10.7	11.3	7.6	3.7	14.7	23.0	46.1	51.3	19.6	10.1
	3-6	12.1	6.1	6.5	3.7	16.2	21.4	50.3	54.6	13.2	13.9
Mechanical analysis	0-6	5.2		3.4		5.8		20.5		45.8	
											19.3

\* Aggregate analysis data for the soil at its field moisture content—shown in table 2a to average approximately 27 per cent, which is the field capacity moisture percentage for this soil.

† Aggregate analysis data for the soil at its air-dry moisture content—found to be approximately 1 per cent.

to study the effect of air-drying upon the distribution of the various aggregate sizes. The soil moisture content at the time of sampling was at or near field capacity. Upon air-drying of a separate portion of these same samples, the soil

moisture content was reduced to approximately 1.5 per cent on the basis of the 105°C. oven-dry weight.

The results of the aggregate analyses of the soil from the differently treated plots, at its field capacity and air-dry moisture content, are contained in table 1. Air-drying has effected a decided increase in the 0.2-0.02-mm. or fine sand fraction with a resulting decrease in the >0.2-mm. and the <0.02-mm. fractions.

Russell (18), in discussing the results of investigations dealing with the effect of air-drying on the water-stability of wet aggregates, points out that, in general, drying increases the stability of small crumbs and decreases the stability of the larger soil crumbs. The type or nature of the soil granule, however, influences the manner of reaction to this dehydrating process. The decrease in the <0.02-mm. or silt and clay fractions upon air-drying and especially in the 0.02-0.002-mm. or silt fraction has been noted by Pavlov (16) and other investigators. Puri and Keen (17) and Henin (8), experimenting with crumbs >0.2m n., found that drying stabilized the structure. This decrease in the percentage of silt and clay upon air-drying probably accounts for the decrease in the dispersion rates of the differently treated soils investigated by Browning (6) and for the increase in microaggregation of Collington sandy loam as investigated by Kolodny and Neal (13).

*Effect of season, soil treatment, moisture content, and depth on aggregation of Hagerstown silt loam surface soil*

Tables 2 and 2a contain the data dealing with seven different soil treatments showing the effect of season, moisture content, and surface soil depth on the percentage of water-stable soil aggregates >0.25 mm. when analyzed at the field-moisture content.

The data in table 2a show the typical decrease in soil moisture content during the hot and often dry months of July and August. Precipitation was 3.08 inches below normal during July and August 1944. Except for these two months, both temperature and precipitation were normal throughout the 13-month period. The gradual decline in soil moisture content beginning at or near field capacity in March and reaching its minimum in August, where for the whole surface layer a subwilting percentage was reached, is representative of the typical drying portion of the soil moisture cycle for this region. Soil moisture content increased with depth of surface layer. The September 30 sampling was made after the beginning of the wetting or replenishing portion of the soil moisture cycle, and with the December and February samplings, frozen gravitational water increased the moisture content of some of the 0-1- and 1-3-inch layers to almost the saturation percentage.

The aggregation data of the soil in its field-moist condition reveal some trends with regard to the relative structural stability of the different surface layers during different parts of the year. At the time of the March, April, May, and June samplings the 1-3- and 3-6-inch surface soil layers possessed a significantly greater aggregation than did the 0-1-inch layer. This difference was most pronounced at the April 17 sampling. One of the principal reasons for the poor

structure in the 0-1-inch layer is the exposure of this unprotected layer to the usual heavy spring rains, the daily occurrence of which is given in figure 1.

TABLE 2

*Seasonal effects on aggregation of Hagerstown silt loam in its field-moist condition under various treatments*

Sampling dates March 17, 1944 to March 16, 1945

TREATMENT*	SOIL DEPTH inches	PERCENTAGE SOIL AGGREGATES > 0.25 MM.											
		Mar. 17	Apr. 17	May 15	Jun. 26	July 24	Aug. 21	Sept. 30	Oct. 30	Nov. 25	Dec. 21	Feb. 3	Mar. 16
Check—un-tilled	0-1	33.5	29.7	35.7	33.7	29.0	37.6	41.0	39.2	39.1	48.8	49.4	32.2
	1-3	51.6	49.2	43.9	37.6	30.6	33.9	43.8	47.9	51.4	44.4	47.4	45.6
	3-6	42.9	43.1	41.6	41.4	29.4	33.4	38.9	45.3	46.1	36.8	38.8	40.8
Check—tilled	0-1	28.1	24.7	32.3	36.9	31.3	36.5	40.2	44.0	49.8	43.6	51.8	39.0
	1-3	48.2	41.1	40.2	47.4	30.4	34.3	43.6	48.2	42.3	45.1	59.9	51.7
	3-6	39.3	38.5	37.8	40.6	31.1	28.9	40.2	49.6	55.7	40.8	45.0	38.1
Manure incorporated	0-1	31.1	34.1	34.5	36.2	33.7	44.5	49.3	52.4	51.6	42.2	57.3	41.3
	1-3	55.1	48.9	45.4	51.9	35.5	44.8	57.8	56.5	59.6	52.0	65.7	54.2
	3-6	52.5	46.8	40.4	45.1	35.9	40.6	57.2	54.0	59.4	48.8	62.8	58.6
Straw incorporated	0-1	28.2	25.4	33.2	43.1	32.8	44.8	47.7	47.4	50.9	45.4	49.5	40.2
	1-3	48.5	44.3	46.1	62.0	32.1	32.9	41.9	52.1	53.2	48.5	59.9	53.4
	3-6	47.3	46.6	47.1	56.2	31.6	34.2	46.4	43.5	55.6	45.3	54.0	36.6
Corn stover incorporated	0-1	29.6	25.4	35.0	31.7	28.1	34.7	40.4	42.5	47.8	41.5	50.6	30.7
	1-3	45.6	39.4	48.4	52.9	27.6	29.1	54.5	57.2	51.4	43.8	50.5	42.4
	3-6	42.2	36.9	43.6	42.8	26.3	27.1	45.6	45.6	48.4	39.3	43.4	31.8
Ryegrass cover and green manure crop	0-1	30.9	36.0	38.8	45.4	35.8	46.7	48.5	53.9	47.6	52.8	53.7	34.1
	1-3	46.8	55.0	45.3	54.3	34.0	45.9	49.3	59.6	57.9	48.7	57.7	45.4
	3-6	52.9	50.4	50.3	48.2	35.8	36.5	49.8	50.0	56.7	43.1	56.4	45.1
800 pounds of 4-12-8 fertilizer broadcast and plowed down	0-1	32.1	32.3	32.4	39.2	29.5	32.4	37.2	43.1	42.1	39.2	51.2	33.9
	1-3	36.1	30.8	38.1	40.4	24.1	26.4	41.5	43.8	42.7	32.4	50.5	41.4
	3-6	38.1	33.2	33.5	41.3	25.5	24.4	39.2	40.6	39.5	33.0	50.3	31.8

\* Except for the untilled check treatment each plot was plowed June 6, at which time the manure, straw, and corn stover applied at the rate of 2.5 tons of dry matter per acre, the ryegrass cover crop, and the fertilizer were incorporated within the surface soil. Each plot was cultivated three times, July 9, August 3, August 27. Corn was planted in 3-by 3-foot hills in each plot on June 9 and harvested September 9. Domestic ryegrass, the one cover crop treatment, was sown at the rate of 20 pounds per acre during the last cultivation, August 27. The 4-12-8 fertilizer applied at the rate of 800 pounds per acre was broadcast and plowed down on June 6.

Spring plowing seems to have effected a temporary increase in the aggregation of the 1-3- and 3-6-inch layers.

By July 24, aggregation was the lowest of any period of the year. The greatest decrease occurred in the 1-3- and 3-6-inch surface layers. Drying seems to have been responsible, in part at least, for this reduction in the percentage of aggregates >0.25 mm. if the data contained in table 1 are applicable to this case. Subsequent wetting and drying, along with cultivation during the equally hot and dry

TABLE 2a

*Field moisture content of soil at each sampling date, in which moist condition the aggregation of the soil was measured as shown in table 2*

Sampling dates March 17, 1944 to March 16, 1945

TREATMENT	SOIL DEPTH inches	PERCENTAGE SOIL MOISTURE*											
		Mar. 17	Apr. 17	May 15	Jun. 26	July 24	Aug. 21	Sept. 30	Oct. 30	Nov. 25	Dec. 21	Feb. 3	Mar. 16
Check—un-tilled	0-1	30.7	26.8	22.2	24.6	5.3	1.8	13.3	19.6	27.9	29.5	44.6	29.0
	1-3	29.5	28.5	26.1	26.3	13.9	6.2	16.8	22.3	22.4	26.5	31.5	26.8
	3-6	32.8	25.6	24.5	27.5	15.5	8.7	16.3	22.8	22.6	26.8	25.4	26.1
Check—tilled	0-1	31.2	27.8	24.7	21.8	5.0	2.1	13.2	18.2	27.2	32.8	45.8	25.7
	1-3	32.5	27.5	24.1	23.3	13.3	6.2	15.7	19.7	22.5	28.5	33.4	26.5
	3-6	33.8	27.4	26.0	25.3	15.1	7.5	16.6	21.6	22.8	25.6	31.6	25.3
Manure incorporated	0-1	32.0	28.6	20.6	16.6	2.1	2.0	13.9	15.8	30.9	38.6	43.4	27.8
	1-3	30.9	30.3	25.5	24.7	12.0	5.8	17.7	19.6	22.7	34.6	31.7	27.4
	3-6	30.5	27.2	26.7	26.3	13.7	7.5	18.2	22.1	23.4	23.4	29.1	26.8
Straw incorporated	0-1	31.2	27.3	26.2	20.2	4.8	2.0	16.4	14.3	29.8	46.7	45.0	27.9
	1-3	32.6	27.1	25.7	23.9	15.2	6.3	17.2	19.9	22.1	37.9	32.1	28.5
	3-6	33.7	28.4	26.8	26.8	16.0	8.4	13.3	22.0	21.0	31.0	30.5	27.3
Corn stover incorporated	0-1	28.1	20.6	13.5	13.0	2.7	1.5	12.8	11.0	29.4	39.9	43.6	23.1
	1-3	29.1	22.8	18.5	19.2	9.8	5.2	16.1	17.4	18.8	36.5	37.2	22.6
	3-6	29.6	23.6	21.0	21.7	10.6	6.9	15.3	19.7	19.3	23.7	31.0	21.2
Ryegrass cover and green manure crop	0-1	30.7	24.7	16.4	16.7	3.6	1.7	12.7	18.5	29.5	34.9	46.8	27.8
	1-3	30.6	23.9	19.9	22.0	12.9	5.8	15.6	19.8	21.4	26.7	35.2	26.8
	3-6	27.8	26.7	24.9	24.3	13.7	6.8	15.9	21.0	21.6	24.4	30.2	25.8
800 pounds of 4-12-8 fertilizer broadcast and plowed down	0-1	25.8	22.3	16.6	18.5	4.7	1.4	13.1	15.6	32.3	27.8	39.0	23.4
	1-3	25.9	23.5	20.6	20.9	10.2	5.6	15.4	17.6	21.6	26.8	31.2	24.3
	3-6	24.7	25.0	22.8	22.4	9.8	6.1	16.0	18.8	20.5	23.6	30.3	23.5

\* On basis of 105°C. oven-dry weight.

month of August, seems to have brought about a slight increase in the percentage of larger aggregates. The September, October, and November samplings showed a very distinct increase in the aggregation of the soil under each of the various treatments. The time interval between the November and December samplings represented a period of freezing and thawing, due to daily and weekly tempera-

ture fluctuations, which may account for the decrease in the percentage of larger aggregates. The February data show a very marked increase in the aggregation of the soil. Throughout January the soil remained frozen at a moisture content ranging from field capacity to almost complete saturation. The formation of large aggregates under these conditions in a soil of this texture agrees with the findings of Jung (12).

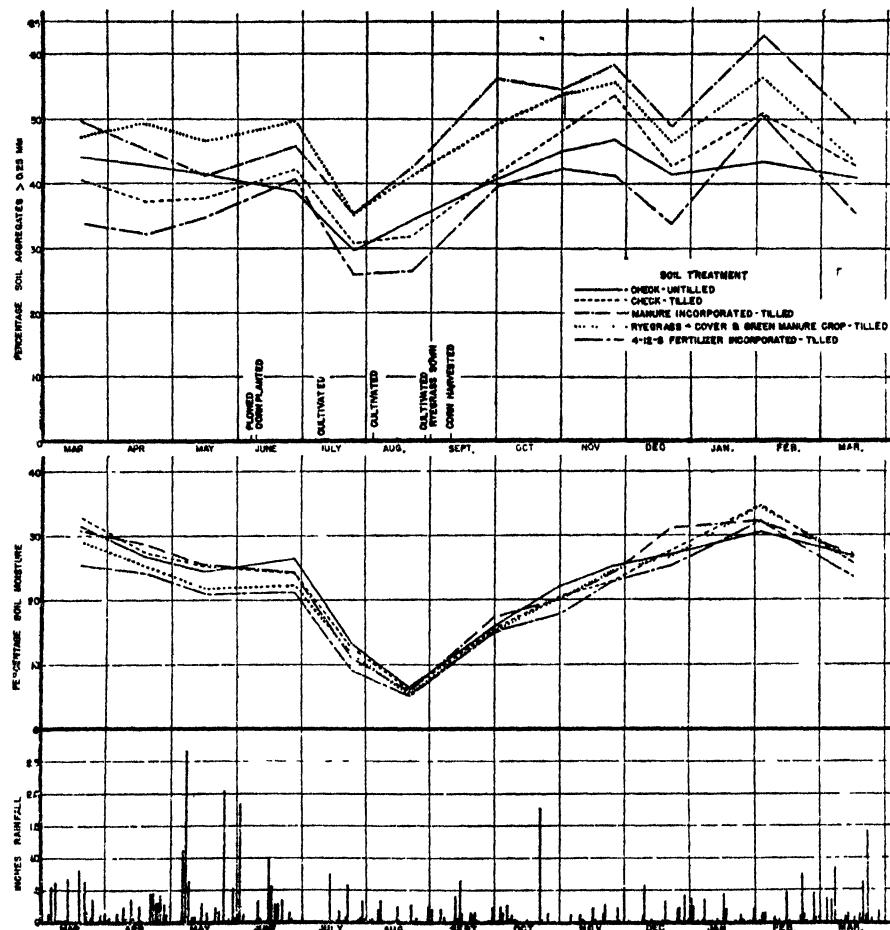


FIG. 1. SEASONAL EFFECTS ON AGGREGATION AND MOISTURE CONTENT OF HAGERSTOWN SILT LOAM SURFACE SOIL, ANALYZED AT FIELD MOISTURE CONTENT

At the March 16, 1945 sampling, by which time the soil had thawed and the snow had disappeared followed by a rise in soil and air temperature, the aggregation increase which occurred during February was found to be only temporary. The percentage aggregation on March 16, 1945 had been reduced to about that which existed on March 17, 1944. The outstanding exception between the aggregation on these two dates was found in the 0-1-inch surface layer, where

aggregation was found to be generally greater at the March 16, 1945 sampling. The 3-6-inch layer, on the other hand, possessed poorer structure than it had a year previous. These differences may be explained by a difference in the type of winter that prevailed previous to each of these sampling dates. Very little snow was present on the soil surface during the winter of 1943-44 with the result that alternate freezing and thawing of the 0-1-inch layer continued throughout the season, but the lower depths remained continually frozen. During the 1944-45 winter, a heavy blanket of snow covered the surface from mid-December to early March. The 0-1-inch layer remained frozen for almost two months, but the lower depths became frozen only very slowly and remained so for perhaps a month.

Figure 1 is a graphic presentation of the variation in the percentage of soil aggregates  $>0.25$  mm. for the whole 0-6-inch layer when analyzed at its field moisture content. It would be inaccurate to suggest that the lines connecting the points obtained by plotting the aggregation percentages represent the actual unvarying gradation in structural condition or soil moisture content. In all probability many fluctuations occur between these sampling dates due to wetting and drying and freezing and thawing. These points, thus, represent the net effects of daily, seasonal, and possibly even annual conditions in each of these plot soils at the particular time when they were sampled. Some recent unpublished data of Fowler and Hide<sup>3</sup> show considerable variation in the aggregation of similarly treated soils sampled at five different dates during November 1943 in Kansas.

#### *Seasonal effects on aggregation and moisture content of Hagerstown silt loam under various mulches*

The data in tables 3 and 3a show the seasonal effects on aggregation and moisture content at different surface soil depths under six different mulches and, for comparative purposes, the untilled check plot.

The data in table 3a show that by August 21 the growing corn crop had reduced the moisture content of most of the plots to within the wilting range. This occurred in those plots where crop growth had been most vigorous. The mulched plots contained a greater amount of soil moisture throughout the year than did the unmulched plot or those in which the same type of mulching material had been incorporated.

Aggregation measurements show that under all the mulches except the sand and gravel the 0-1-inch surface layer was in the best structure. The percentage of aggregates  $>0.25$  mm. decreased with increasing surface soil depth. This trend had become apparent by 1942. The results of a structural analysis of all the plots in replicate treatment were given in a previous publication (2). Stephenson and Schuster (19) have shown a similar decrease in the percentage of aggregates  $>1.0$  mm. under a straw and a trash mulch with successively deeper surface soil layers. The effect of a decrease in moisture content, which reached a mini-

<sup>3</sup> Fowler, E. B., and Hide, J. C. Unpublished data. Kansas Agricultural Experiment Station. 1944.

mum in August, is reflected in a decrease in the number of the larger aggregates. The greatest amount of aggregation under the mulches was found during September and October. This increased aggregation in the 0-1- and 1-3-inch layers

TABLE 3

*Seasonal effects on the aggregation of Hagerstown silt loam in field-moist condition under various mulches*

Sampling dates March 17, 1944 to March 16, 1945

TREATMENT*	SOIL DEPTH <sup>a</sup>	PERCENTAGE SOIL AGGREGATES > 0.25 MM.											
		Mar. 17	Apr. 17	May 15	Jun. 26	July 24	Aug. 21	Sept. 30	Oct. 30	Nov. 23	Dec. 21	Feb. 3	Mar. 16
	inches												
Cheek—un-tilled	0-1	33.5	29.7	35.7	33.7	29.0	37.6	41.0	39.2	39.1	43.8	49.4	32.2
	1-3	51.6	49.2	43.9	37.6	30.6	33.9	43.8	47.9	51.4	44.4	47.4	45.6
	3-6	42.9	43.1	41.6	41.4	29.4	33.5	38.9	45.3	46.1	36.8	38.8	40.8
Manure mulch	0-1	65.4	61.8	57.2	41.1	57.7	59.3	68.3	70.7	63.6	62.9	60.2	55.0
	1-3	53.7	44.9	47.5	41.8	33.3	44.8	51.1	58.1	61.5	56.3	53.2	51.3
	3-6	45.1	37.6	37.6	30.0	25.9	34.8	45.1	42.1	32.2	32.0	31.4	26.9
Straw mulch	0-1	51.2	42.7	42.9	53.6	46.3	35.7	58.2	65.3	58.3	55.5	48.7	50.2
	1-3	40.5	32.5	45.7	42.8	46.3	29.6	48.0	51.8	53.2	54.3	47.9	49.2
	3-6	42.0	37.1	36.1	36.9	42.3	30.1	39.0	45.8	42.5	39.0	32.2	31.4
Corn stover mulch	0-1	59.3	55.2	55.0	50.5	47.1	42.5	66.7	73.3	56.7	55.6	57.1	43.3
	1-3	48.2	41.5	41.3	46.3	41.5	31.4	53.9	61.0	54.6	53.0	47.7	37.2
	3-6	39.6	31.1	30.9	33.9	32.1	28.8	51.5	56.3	38.9	31.7	31.1	24.5
Oak leaves mulch	0-1	58.5	50.3	59.1	53.4	48.5	47.5	64.9	55.0	58.6	63.9	66.6	53.3
	1-3	34.2	50.9	49.4	51.4	47.2	37.7	58.9	50.1	52.2	54.3	57.2	50.3
	3-6	40.4	38.0	36.9	39.7	46.8	34.5	47.7	44.2	34.4	38.5	38.2	29.2
Pine needles mulch	0-1	55.5	48.1	53.9	64.5	55.1	46.9	71.7	64.6	61.4	65.6	63.0	57.6
	1-3	56.4	50.5	50.6	55.1	50.0	36.7	54.3	52.9	47.7	58.5	54.7	52.9
	3-6	51.0	40.4	36.0	37.0	34.3	28.5	49.1	42.3	35.8	40.6	35.6	32.4
Sand and gravel mulch	0-1	32.5	30.7	31.4	33.2	30.0	23.0	41.0	45.2	42.0	34.4	43.5	28.8
	1-3	42.6	40.2	41.4	41.6	36.2	30.1	46.6	48.3	47.9	39.9	47.5	32.6
	3-6	43.8	42.1	32.7	39.2	32.2	30.5	42.4	41.7	37.8	32.9	37.6	29.4

\* Uniform cropping and weeding were followed throughout. Mulching material was added at the rate of 2.5 tons of dry matter per acre to each plot on June 6. Corn was planted in 3- by 3-foot hills on each plot June 9 without previous tillage. Weeds were removed by pulling and scraping on June 6, July 9, August 3, August 27. The corn was harvested on September 9. The soil in each plot remained undisturbed throughout the year.

seemed to be rather well maintained under the manure, oak leaves, and pine needles throughout the winter.

The pronounced decrease in aggregation that occurred following the annual

June application of the manure mulch may have been caused by the leaching of ammonia from the fresh manure which, in the presence of an excess of moisture, may have resulted in a temporary dispersion of some of the less stable aggregates.

TABLE 3a

*Field moisture content of soil at each sampling date, in which moist condition the aggregation of the soil was measured as shown in table 3*

Sampling dates March 17, 1944 to March 16, 1945

TREATMENT	SOIL DEPTH inches	PERCENTAGE SOIL MOISTURE*											
		Mar. 17	Apr. 17	May 15	Jun. 26	July 24	Aug. 21	Sept. 30	Oct. 30	Nov. 25	Dec. 21	Feb. 3	Mar. 16
Check—un-tilled	0-1	30.7	26.8	22.2	24.6	5.3	1.8	13.3	19.6	37.9	39.5	44.6	29.0
	1-3	29.5	28.5	26.1	26.3	13.9	6.2	16.8	22.3	22.4	26.5	31.5	26.8
	3-6	32.8	25.6	24.5	27.5	15.5	8.7	16.3	22.8	22.6	26.8	25.4	26.1
Manure mulch	0-1	38.8	33.5	28.7	44.7	34.9	10.6	20.0	31.0	31.2	33.7	39.7	42.6
	1-3	32.5	28.6	26.9	38.0	15.5	11.1	20.1	25.5	26.9	28.7	27.7	31.6
	3-6	31.6	28.1	26.1	32.2	12.3	9.5	17.6	24.0	26.3	27.6	24.6	26.2
Straw mulch	0-1	37.3	36.9	32.0	37.4	25.3	15.7	24.7	28.6	31.8	30.5	32.5	35.5
	1-3	35.1	32.1	31.2	33.5	24.4	15.9	23.2	27.4	28.9	29.5	32.1	28.4
	3-6	36.3	30.7	31.3	20.7	21.4	16.7	22.5	26.9	27.2	28.4	27.3	25.4
Corn stover mulch	0-1	32.4	30.9	30.4	28.6	15.8	8.9	21.5	23.1	26.4	27.2	35.0	28.3
	1-3	28.6	25.8	27.4	27.0	16.4	9.4	19.6	21.9	23.2	25.7	31.6	25.9
	3-6	29.7	27.3	27.4	26.8	15.6	10.5	18.8	21.4	23.2	24.8	25.1	24.4
Oak leaves mulch	0-1	33.6	31.4	28.4	33.7	24.0	16.5	25.0	28.4	29.7	29.3	33.8	37.1
	1-3	30.9	28.6	27.1	30.0	22.1	14.8	22.5	25.4	26.8	27.4	32.2	27.9
	3-6	31.8	27.9	27.6	29.0	21.4	13.7	22.0	23.6	25.0	24.5	23.8	25.4
Pine needles mulch	0-1	35.3	29.8	28.3	32.4	13.0	8.2	20.2	25.5	25.5	27.4	33.6	33.8
	1-3	33.0	28.7	27.4	30.1	16.3	10.3	20.1	23.6	24.5	25.2	28.1	29.6
	3-6	32.3	28.4	27.8	28.9	15.6	10.4	19.9	23.1	24.2	24.8	25.2	25.9
Sand and gravel mulch	0-1	28.6	24.2	21.4	21.6	11.5	6.0	17.4	16.2	17.9	23.9	33.1	24.1
	1-3	29.3	25.7	25.1	24.4	15.3	8.5	18.9	22.7	21.7	24.1	32.7	27.2
	3-6	30.9	27.6	26.1	24.5	16.9	10.0	19.6	23.5	23.9	24.3	24.5	26.7

\* On basis of 105°C. oven-dry weight.

*Effect of air-drying on aggregation of Hagerstown silt loam at different sampling periods throughout year*

It has been a common practice to air-dry the field soil sample before determining its aggregate condition. This has been done because it was recognized that aggregation varied at least in some degree with soil moisture content, hence, it seemed logical to bring all of the samples to a common moisture content, which was quite generally the air-dry condition. To determine the effect of air-drying

TABLE 4

*Aggregation of Hagerstown silt loam analyzed after air-drying*

Sampling dates March 17, 1944 to March 16, 1945

TREATMENT	SOIL DEPTH <i>inches</i>	PERCENTAGE SOIL AGGREGATES > 0.25 MM.											
		Mar. 17	Apr. 17	May 15	Jun. 26	July 24	Aug. 21	Sept. 30	Oct. 30	Nov. 25	Dec. 21	Feb. 3	Mar. 16
Check—un-tilled	0-1	29.0	21.4	30.1	38.8	34.2	42.3	38.2	32.6	27.1	33.8	30.7	26.5
	1-3	30.8	28.5	41.1	40.3	38.7	39.3	40.2	39.1	32.3	33.0	33.6	28.2
	3-6	31.0	26.7	34.0	42.1	44.9	35.5	41.2	36.8	31.7	27.5	28.2	30.4
Check—tilled	0-1	24.7	22.1	27.9	40.8	41.9	43.7	30.0	38.6	34.9	31.2	28.3	26.7
	1-3	29.6	31.4	32.3	41.0	40.9	40.2	42.2	39.5	30.5	30.3	29.9	29.0
	3-6	29.9	27.8	34.9	35.2	34.0	35.3	34.2	37.1	30.3	26.3	24.6	26.8
Manure mulch	0-1	58.9	72.9	76.5	76.6	81.9	75.9	62.7	65.3	59.0	61.8	50.1	60.7
	1-3	49.0	53.3	63.7	60.9	63.6	61.5	53.1	48.4	45.7	45.3	44.6	57.1
	3-6	38.1	39.2	54.8	48.8	53.4	52.3	41.6	39.8	37.7	33.0	41.6	45.3
Manure incorporated	0-1	32.2	27.6	32.1	46.2	46.3	55.4	45.2	37.4	34.2	31.3	31.9	26.7
	1-3	43.4	42.2	44.1	50.3	50.9	51.3	54.1	48.7	43.2	36.7	36.6	38.2
	3-6	55.7	46.7	44.1	43.5	46.3	51.7	50.9	43.2	43.3	35.1	42.8	38.0
Ryegrass cover crop	0-1	30.1	29.1	34.3	45.8	43.9	52.6	44.2	42.9	41.0	29.7	33.2	26.6
	1-3	43.3	46.4	40.8	46.0	45.0	52.0	46.4	42.8	39.0	36.9	37.6	30.5
	3-6	44.1	43.4	47.6	49.0	46.2	43.5	47.5	41.6	37.4	32.0	37.9	32.7
4-12-8 fertilizer	0-1	28.0	23.0	26.6	31.6	36.2	37.8	31.3	35.3	33.3	25.2	29.1	24.2
	1-3	28.4	33.7	29.2	32.0	32.9	32.0	32.8	33.1	29.8	25.1	27.8	27.2
	3-6	24.5	30.1	29.4	29.2	34.5	32.6	28.7	31.7	30.3	25.5	28.2	27.7

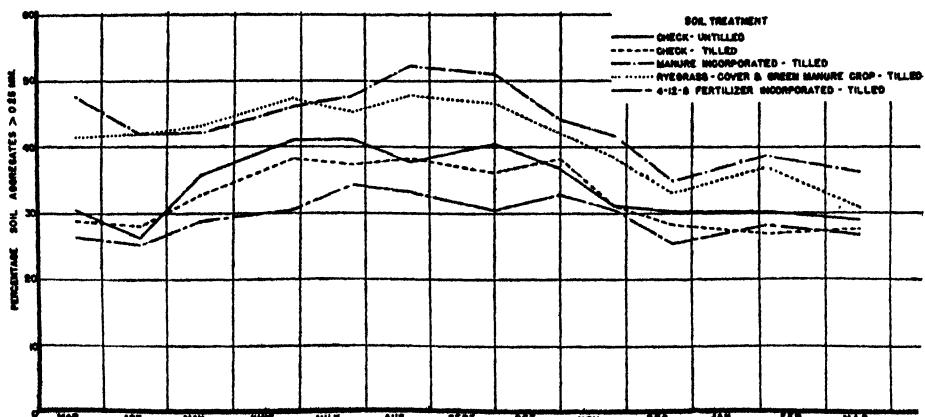


FIG. 2. SEASONAL EFFECTS ON AGGREGATION OF HAGERSTOWN SILT LOAM SURFACE SOIL, ANALYZED AFTER AIR-DRYING IN THE LABORATORY

TABLE 5

*Seasonal effect on aggregate size distribution in manured and unmanured Hagerstown silt loam analyzed in field-moist and air-dry conditions*

SAMPLING DATE	TREATMENT	PERCENTAGE SOIL AGGREGATES											
		> 2.0 mm.		2.0-1.0 mm.		1.0-0.2 mm.		0.2-0.02 mm.		0.02-0.002 mm.			
		Field-moist*	Air-dry†	Field-moist	Air-dry	Field-moist	Air-dry	Field-moist	Air-dry	Field-moist	Air-dry		
1944													
Mar. 17	Manure incorporated.....	12.0	8.5	17.2	4.0	26.4	31.4	34.5	51.7	9.5	4.3	0.4	0.1
	Unmanured tilled check..	8.7	8.4	11.9	2.3	28.7	20.6	40.6	62.0	9.3	6.7	0.8	0
Apr. 17	Manure incorporated.....	10.2	8.4	9.8	4.9	29.5	29.4	35.8	51.8	14.0	5.5	0.7	0
	Unmanured tilled check..	9.8	9.3	5.8	3.6	25.7	19.5	42.6	58.7	14.8	8.7	1.3	0.2
May 15	Manure incorporated.....	11.0	8.9	12.4	4.7	24.7	31.3	40.9	48.8	10.4	5.9	0.6	0.4
	Unmanured tilled check..	9.8	8.8	6.6	2.9	24.2	20.9	45.8	58.5	12.9	8.4	0.7	0.5
Jun. 26	Manure incorporated....	17.8	9.6	13.7	6.6	21.7	34.4	37.3	44.8	8.9	4.0	0.6	0.6
	Unmanured tilled check..	11.3	7.6	9.2	4.9	27.5	28.8	41.5	51.4	9.5	6.8	1.0	0.5
July 24	Manure incorporated.....	11.8	10.9	4.2	6.9	20.3	33.9	54.5	42.2	8.7	5.4	0.5	0.7
	Unmanured tilled check..	6.7	9.3	4.0	3.0	20.3	29.2	58.6	50.6	9.8	7.1	0.6	0.8
Aug. 21	Manure incorporated.....	14.4	11.4	7.8	6.7	23.4	33.5	45.9	42.5	8.1	5.7	0.4	0.2
	Unmanured tilled check..	8.7	11.1	3.8	3.8	22.2	25.9	55.5	51.6	9.6	7.5	0.20	0.1
Sept. 20	Manure incorporated.....	17.0	12.0	12.5	8.6	29.9	35.2	34.0	39.6	6.4	4.3	0.2	0.3
	Unmanured tilled check..	7.2	9.2	3.8	2.7	34.2	31.6	45.5	50.1	8.9	6.0	0.4	0.4
Oct. 30	Manure incorporated.....	17.7	8.1	10.5	5.7	28.5	35.3	34.9	44.5	8.4	6.3	0	0.1
	Unmanured tilled check..	11.0	7.5	6.6	3.4	31.1	28.9	41.2	52.2	9.5	7.9	0.6	0.1
Nov. 25	Manure incorporated.....	22.3	11.0	14.8	5.5	24.1	29.0	28.6	48.0	9.6	6.3	0.6	0.2
	Unmanured tilled check..	12.0	8.8	9.0	2.8	31.7	20.4	36.9	60.4	10.1	7.0	0.3	0.6
Dec. 21	Manure incorporated.....	12.9	6.8	12.3	4.2	28.3	26.3	32.5	54.1	13.5	8.4	0.5	0.2
	Unmanured tilled check..	5.9	7.0	9.0	2.4	31.1	21.3	38.0	56.5	15.0	12.3	1.0	0.5
1945													
Feb. 3	Manure incorporated.....	18.4	7.8	18.9	4.3	29.2	24.8	24.4	54.2	8.5	8.6	0.6	0.3
	Unmanured tilled check..	9.3	6.2	12.3	3.3	38.4	21.3	30.3	56.2	8.8	12.4	0.9	0.6
Mar. 16	Manure incorporated.....	15.1	8.9	15.6	4.7	24.2	26.1	29.1	50.1	15.3	9.8	0.8	0.4
	Unmanured tilled check..	11.3	8.4	12.9	2.3	28.1	18.7	30.4	58.0	16.1	12.1	1.2	0.6
	Mechanical analysis 1-3- inch surface soil layer		5.7		2.6		6.1		21.1		46.2		18.3

\* Field-moist data are for the 1-3-inch layer of surface soil analyzed at its field-moisture content. Actual percentage moisture of this layer at each sampling period is shown on table 2a.

† Air-dry data are for the 1-3-inch layer of surface soil analyzed at its air-dry moisture content. The percentage moisture on the 105°C. over-dry basis of each air-dried sample ranged from 1 to 2 per cent.

on the percentage of aggregates  $>0.25$  mm. in soils of different moisture contents at the time of sampling, the soils from six different treatment plots were analyzed after air-drying. The soils in this condition contained 1 to 2 per cent moisture on the  $105^{\circ}\text{C}$ . oven-dry basis. The results of this study are given in table 4. The results of the analysis of the soil that was air-dried are quite different from the results of a portion of the same soil sample that was analyzed at its field-moist condition as shown in tables 2 and 3. Air-drying had obliterated or at least modified the wide differences in the aggregate condition of the same soil as it was sampled and analyzed at different moisture contents throughout the year. This may be further emphasized by a study of the aggregation curves of the field-moist and air-dried soils shown in figures 1 and 2, in which latter figure the results of the aggregate analysis of the whole 0-6-inch air-dried surface soil are plotted. The pronounced decrease in aggregation in July for the field-moist soils illustrated in figure 1 is lacking in figure 2. In fact, air-drying a portion of both the July and August samples has effected an increase in the proportion of larger soil aggregates.

To study the effect of season on the aggregate size distribution, the 1-3-inch surface layers of two differently treated soils were selected for complete aggregate analysis in their field-moist and air-dry conditions. The results are given in table 5. Here again is illustrated the general tendency for the percentage of larger soil aggregates to decrease upon air-drying whether in the field or upon removal to the laboratory. For this particular soil the tendency is for air-drying to effect an increase in the 1.0-0.2-mm. and 0.2-0.02-mm. or medium and fine sand fractions. The analyses of the July and August samples given in table 5 may assist in explaining the difference in the shapes of the field-moist and air-dry soil-aggregation curves in figures 1 and 2. Analysis of the July and August samples in their field condition show that natural wetting and drying action during the midsummer months resulted in a breaking down of the larger sized aggregates, which existed during periods of higher soil moisture content, into granules of the 0.2-0.02-mm. or fine sand dimension group. Air-drying portions of these same samples effected some aggregating action, slight though it may have been, which resulted in an increase in the next larger size fraction, the 1.0-0.2-mm. or medium sand group. A considerable proportion of this increase is, thus, included in the  $>0.25$ -mm. fraction shown in table 4 and figure 2 for the July and August samplings.

#### DISCUSSION

The experimental results of this study demonstrate the variable and dynamic nature of soil structure as measured by the aggregate condition of field soils.

A true explanation of all the causes for the variation in the aggregation of this or any other soil under actual field conditions on an accurate quantitative basis, of course, does not exist. The multiplicity of factors involved, especially under field conditions, makes the obtaining of accurate quantitative information involving this phenomenon a very difficult task. Efforts have been made to determine the extent to which certain soil qualities affect the aggregation of a particular soil or of an artificial mixture of several soil-like constituents. Krause (14), Russell

(18), and Baver (5) have presented excellent discussions of the factors involved in the aggregation of soils.

Although moisture content is undoubtedly an important factor in aggregation, the experimental results of this study do not suggest any simple linear relationship. The fluctuation in the aggregation of this soil is the result of numerous factors.

Alternate wetting and drying resulting in build-up and breakdown of soil granules is responsible in part for the continuous increase in the percentage of aggregates  $>0.25$  mm. when analyzed in the field-moist condition from July until November.

The difference in the water stability of the aggregates in the air dry and field-dry soil at the August 21 sampling may be due to an irreversible or at least very slowly reversible change in the colloids of iron and aluminum following air-drying in the laboratory at the usual high summer temperature. The laboratory air-dried August samples possessed the greatest amount of aggregation contained in any of the air-dried samples throughout the year, and the same field-dry August samples, possessing a minimum of moisture, contained next to the least percentage of water-stable aggregates. The additional dehydration of the colloids in the soil at this moisture content upon drying in the laboratory may have effected a cementing of the structural particles, resulting in aggregates of increased size and stability. The same field-dry samples, on the other hand, because of their decreased moisture content, may have lost the maximum effect of moisture linkage between the soil particles or aggregates except in the case of a few very stable granules.

At least two reasons may be advanced to account for the decreased aggregation of the December samples. The increased moisture content may have effected such a lengthening, with a resulting weakening, of the moisture linkage or colloidal binding in the previously water-stable granules that a measurable amount of dispersion had taken place. Alternate freezing and thawing may also have had its effect. Baver (5) has observed that "the aggregation produced by several freezings and thawings decreases rapidly as the number of freezings increases. This is particularly true of silty soils that are low in organic matter." He further asserted that "similar to the aggregates produced by alternate wetting and drying those that are formed by freezing and thawing are more or less temporary unless sufficient organic matter is present to stabilize them."

Thus is introduced the rôle of organic matter in structural stability. It will have been noted throughout the study of the results of this investigation that those soils to which organic matter had been added maintained a consistently greater percentage of aggregates  $>0.25$  mm. regardless of when or in what condition the soil was sampled. This stabilizing action of organic matter has been noted by many investigators.

Because of a lack of replication, no statistical significance can be claimed for the differential effects of soil treatment. In the original selection of the plots, however, for the purpose of obtaining the maximum uniformity, those in check treatments were, if anything, given a slight advantage especially in the matter of

soil organic matter content. Thus, after 6 years of the same individual cultural treatment, the fact that the soils to which organic matter has been added have maintained a consistently greater aggregation may be considered something less than purely accidental.

The maintenance of the soil in a continuously frozen condition through January, when the mean daily air temperature was 17°F., resulted in an increased aggregation. This high amount of aggregation was not maintained after the soil thawed. The decrease in aggregation, as shown by both of the March samplings, was much less pronounced in the soils to which organic matter had been added.

The effect of mulching on the aggregation of the surface soil which had no tillage was to increase markedly the percentage of aggregates  $>0.25$  mm., especially in the 0-1-inch layer and, to a lesser extent, in the 1-3-inch layer. The presence of actively decomposing mulching material effected an unmistakable alteration in the structure of the soil. This is immediately apparent upon observation of the surface conditions under the organic mulches. Biological activity in the 0-1- and 1-3-inch surface soil layers under these mulches is great, as evidenced by the large number of earthworm casts and the intricate network of mycelial strands surrounding and connecting the soil granules. The contrast in the aggregation of the 0-1-inch soil layers under these mulches and when this same layer is allowed to remain exposed is most marked. The exposed surface layer of the untilled check plot remained dispersed and panlike and lacked any visible evidence of biological activity. The effect of the surface mulches on the 3-6-inch layer after 5 years of continuous treatment is very slight when compared with the 3-6-inch layer in the untilled check plot.

Within the surface soil layer of the unmulched soils, the 1-3-inch layer usually possessed the greatest percentage of water-stable aggregates. The 3-6-inch layer possessed the next greatest, and the surface inch the least percentage of aggregates  $>0.25$  mm. It appears from a study of the trends in aggregation within these three surface layers that the portion of the surface soil which is free from the effects of mechanical dispersion due to rainfall, machinery, or animals, where moisture content and aeration is sufficient for maximum biological activity, where the maximum effects of alternate wetting and drying are possible, and where alternate freezing and thawing is reduced to a minimum, will possess the greatest aggregation. It is within the 1-3-inch surface layer of the average agricultural soil that these conditions are most likely to prevail during most of the year. A shift in maximum aggregation to the 0-1- or 3-6-inch level may, of course, occur as one of these factors such as increased moisture content or continued freezing persists in either of these two other layers.

In the discussion of the bearing this work may have upon the quantitative significance of aggregation studies, it may be concluded that the conditions under which or following which a determination of the amount of aggregation is made are important. Although the relative order of the difference in aggregation between differently treated soils may not have been altered, soil moisture conditions together with the manner of pretreatment alone can greatly alter the measured amount of aggregation as determined by the present method of analysis.

## SUMMARY

Aggregation studies were conducted on plots of Hagerstown silt loam surface soil which had been subject to 5 years of each of the following annual treatments: Manure, straw, corn stover, a ryegrass cover crop, and a 4-12-8 fertilizer were incorporated in the surface soil. Manure, straw, corn stover, oak leaves, pine needles, and sand and gravel were applied as surface mulches. For comparative purposes, check treatments were included in which no soil amendments were used. The soil in one instance was plowed and cultivated, and in the other the soil was kept bare and untilled. Each plot was sampled at monthly intervals beginning March 17, 1944 and ending March 16, 1945. Soil temperature and moisture percentages were determined at each sampling. Aggregate analyses were made on the 0-1-, 1-3-, and 3-6-layers of the soil at its actual field moisture content and after air-drying in the laboratory.

Soil structure, as measured by the percentage of the soil aggregated into particles  $>0.25$  mm. in diameter on the basis of 105°C. oven-dry weight, was found to be subject to wide seasonal variation.

When analyzed in its field-moist condition, the soil possessed a minimum percentage of water-stable aggregates during the hot, dry midsummer period (July and August) and a maximum in the late autumn (November) and also during the late winter (February).

Soil moisture content was closely related to the amount and size of water-stable aggregates when the soil was analyzed in its field-moist condition. The effect of soil moisture content was modified, however, by seasonal conditions such as alternate wetting and drying during the summer and by alternate freezing and thawing, which effected a large but temporary increase in aggregation during the late winter.

Air-drying in the laboratory effected a marked change in the size and quantity of water-stable aggregates as compared with the aggregate distribution in the soil at its actual field moisture content. When analyzed in the air-dry condition the soil possessed a maximum percentage of water-stable aggregates during the months of July, August, and September and a minimum during the winter and early spring months.

Aggregation varied markedly in the different layers of surface soil under the different treatments. Aggregation was greatest in the 1-3-inch surface layer of the tilled soils in which different materials were incorporated, whereas the 0-1-inch layer possessed the least quantity of the larger aggregates. Aggregation of the 3-6-inch surface layer was generally slightly less than that of the 1-3-inch layer. The 0-1-inch layer under the organic material mulches was most highly-aggregated, the 1-3-inch layer was somewhat less aggregated, and the 3-6-inch layer remained almost wholly unaffected by the presence of the surface mulch.

## REFERENCES

- (1) ALDERFER, R. B., AND MERKLE, F. G. 1941 The measurement of structural stability and permeability and the soil treatment upon these properties. *Soil Sci.* 51: 201-212.

- (2) ALDERFER, R. B., AND MERKLE, F. G. 1943 The comparative effects of surface application vs. incorporation of various mulching materials on structure, permeability runoff and other soil properties. *Proc. Soil Sci. Soc. Amer.* 8: 39-86.
- (3) ANDRIANOV, P. I. 1932 Comparative evaluation of the stability of arable land structure in natural conditions. *Proc. 2nd Internat. Cong. Soil Sci., Comn.* I, 1: 59.
- (4) APSITS, J. 1936 Der Einfluss künstlicher und natürlicher Factoren auf die Struktur des Bodens. *Bodenk. u. Pflanzenernähr.* 42: 1-35.
- (5) BAVER, L. D. 1940 *Soil Physics.* John Wiley and Sons, New York.
- (6) BROWNING, B. M. 1937 Changes in erodibility of soils brought about by the application of organic matter. *Proc. Soil Sci. Soc. Amer.* 2: 85-96.
- (7) COLE, R. D. 1939 Soil macrostructure as affected by cultural treatments. *Hortgardia* 12: 429-472.
- (8) HENIN, S. 1936 Étude sur la regeneration des "elements structuraux" de sols de limon. *Ann. Agron.* 6: 455-472.
- (9) HENIN, S. 1938 Étude physico-chimique de la stabilité structural des terres. *Monog. Natl. Center Agron. Res.*, pp. 59-64. Paris.
- (10) HENIN, S. 1939 L'influence des facteurs climatiques sur la stabilité structurale des sols de limon. *Ann. Agron.* 9: 301-311.
- (11) JEFFRIES, C. D., AND WHITE, J. W. 1937 Some mineralogical and chemical characteristics of a Hagerstown soil profile. *Proc. Soil Sci. Soc. Amer.* 2: 133-142.
- (12) JUNG, E. 1931 Untersuchungen über die Einwirkung des Frostes auf den Erdboden. *Kolloidchem. Beihefte* 32: 320-373.
- (13) KOLODNY, L., AND NEAL, O. R. 1941 The use of micro-aggregation or dispersion measurements for following changes in soil structure. *Proc. Soil Sci. Soc. Amer.* 6: 91-95.
- (14) KRAUSE, M. 1931 Russian researches into the structure of soils. *Landw. Jahrb.* 73: 601-690.
- (15) MERKLE, F. G. 1934 Base exchange studies on the Pennsylvania Jordan field plots. *Soil Sci.* 38: 37-64.
- (16) PAVLOV, G. I. 1932 Aggregatenanalyse und Aggregatenzusammensetzung des Bodens. *Proc. 2nd Internat. Cong. Soil Sci., Comn.* I, 1: 179-193.
- (17) PURI, A. N., AND KEEN, B. A. 1925 The dispersion of soil in water under various conditions. *Jour. Agr. Sci.* 15: 147-161.
- (18) RUSSELL, E. W. 1938 Soil structure. *Imp. Bur. Soil Sci. Tech. Commun.* 39.
- (19) STEPHENSON, R. W., AND SCHUSTER, C. E. 1945 Effect of mulches on soil properties. *Soil Sci.* 59: 219-230.
- (20) TUILIN, A. F. 1928 Questions on soil structure: II. Aggregate analysis as a method for determining soil structure. *Perm. Agr. Exp. Sta. (USSR) Div. Agr. Chem.* 2: 77-122.

# EFFECT OF THE NATURE OF EXCHANGEABLE BASES ON SOIL POROSITY AND SOIL-WATER PROPERTIES IN MINERAL SOIL

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The effect of exchangeable bases on the general properties of the soil, and especially on its colloidal part, has already been described by several research workers, including the writer (8, 10). The aim of this paper is to record some soil-water properties of subtropical mineral soils as related to the total quantity and the mono-bivalent Na/Ca ratios of the exchangeable bases.

The soil types under review may be found among the subtropical soils, the genesis and composition of which were discussed in a previous paper (9). The similarity of the chemical composition of the soil profile was noted, and the differences of structure in the subsequent soil layers, resulting from the distribution and degree of saturation of colloidal clay and the depth of horizons, were described.

Four cations, K, Na, Ca, and Mg, were found to be absorbed by the colloidal clay portion of the soils, but they do not play an equal role in building up the internal soil structure. K is present in the soil samples only in very small quantities and may therefore be disregarded for our purposes. As for Mg, it was found that the degree of saturation of the soil colloidal clay is about the same for all layers (9, p. 6), and this cation has, therefore, no great influence on the properties of internal soil structure. The ions most responsible for the physicochemical changes of the soil are Na and Ca, wide differences in their degree of hydration (molecules of water to one equivalent of cation, for  $\text{Na}^+ = 66$  and for  $\text{Ca}^{++_2} = 14$ ) having been established (2, p. 81; 13). Thus a correlation should be sought between different soil-water and structure properties and the Na/Ca ratios.

## SOIL-WATER PROPERTIES OF SOILS WITH LOW CONTENT OF MONOVALENT EXCHANGEABLE CATIONS AND INCREASING Na/Ca RATIOS

For the study of soil-water properties of soils with a low content of exchangeable monovalent cations, samples of gray soils in the northern part of the Jordan Valley were selected. This series of soils belongs to the sedimentary deposits of the Glacial Jordan Salt Lake, the soluble salts of which were thoroughly leached in the postglacial period. The origin of these soils and the geological and physicochemical conditions of their formation have been described elsewhere (9). The soil samples investigated were taken from four layers, one below the other, each 25 cm. in depth. They were passed through a 2-mm. sieve and air-dried. Chemical analysis established the presence of an average of 40 per cent  $\text{CaCO}_3$ , which increased slightly with increasing depth. Constant amounts of  $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$ , and  $\text{MgO}$  were found in all layers. The percentage of monovalent replaceable cations was low and fluctuated between 5.6 and 8.3 (table 2).

*Upward and downward movement of water*

The capillary upward movement was tested by the usual devices in glass tubes filled, under similar conditions, with powdered soil and immersed in jars of distilled water. Figure 1 represents the results obtained after measuring the rise of water in correlation with the depths of the layers. Curves plotted for subsequent layers run parallel, and the degree of capillary rise increases with the depth of soil sample.

The downward movement of the water in the same sample was checked by observing the flow of distilled water through soil columns of equal height and weighing 150 gm. on the dry basis. The recorded figures (table 1) show the time re-

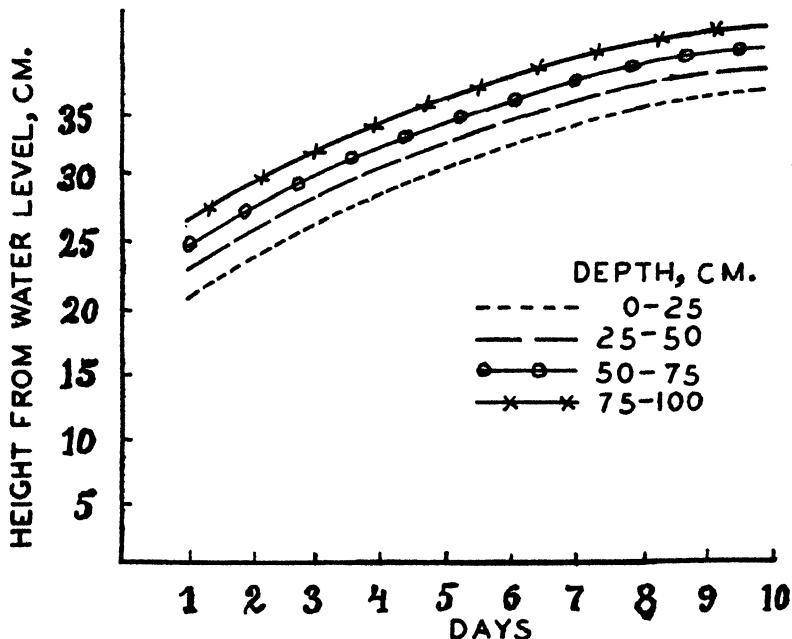


FIG. 1. CAPILLARY RISE OF MOISTURE IN GRAY SOIL OF THE NORTHERN PART OF THE JORDAN VALLEY

quired to fill a graduated 50-cc. flask with the drainage water flowing through the soil column. Table 1 shows that the drainage capacity of the soil increases with depth.

*Exchangeable bases, porosity, internal surface area, and water movement in soil*

The exchangeable bases and the internal surface area were examined in the soil samples with the object of establishing the relation between soil-water properties and the state of the colloidal fraction of the soil. The exchangeable bases were tested by the Hissink method for soils rich in  $\text{CaCO}_3$  (6). The internal surface was measured by the method of Mitscherlich (11), who used the absorption of water vapor from a 10 per cent solution of  $\text{H}_2\text{SO}_4$  by dry soil as a measure of hy-

hygroscopicity ( $Wh$ ), that is, the molecular water film covering the soil particles. This method, at first disputed, was later revived by American investigators, who came to the conclusion that it provides a fair estimate of total colloidal content. Multiplying the hygroscopicity figure ( $Wh$ ) by 40.6 gives the surface in square meters for 1 gm. of soil, according to Rodenwald (4). Recent investigations have shown that the formula of Rodenwald has no absolute value for determining the surface of colloidal matter, but it still retains some importance where relative figures are required.

The data in tables 1 and 2 show that, with increasing depth, the following

TABLE 1

*Downward movement of water through columns of gray soil from the northern part of the Jordan Valley*

DEPTH cm.	TIME REQUIRED TO FILL 50-CC. FLASK WITH DRAINAGE WATER		
	First portion of water	Second portion of water	Third portion of water
0-25	1 hour 53 minutes	2 hours 7 minutes	2 hours 18 minutes
25-50	1 hour 10 minutes	1 hour 16 minutes	1 hour 12 minutes
50-75	1 hour	1 hour	1 hour
75-100	53 minutes	55 minutes	50 minutes

TABLE 2

*Replaceable cations, hygroscopicity, internal surface, specific gravity, and porosity in gray soil of the northern part of the Jordan Valley*

DEPTH	TOTAL CATIONS IN 100 GM. DRY SOIL	MONOVALENT CATIONS, PERCENTAGE OF TOTAL	Na/Ca RATIO*	HYGROSCOPICITY	INTERNAL SURFACE 1 GM. DRY SOIL	APPARENT SP. GR.	REAL SP. GR.	TOTAL POROSITY	CAPILLARY POROSITY	NON-CAPILLARY POROSITY
cm.	m.e.			per cent†	sq. m.			per cent	per cent	per cent
0-25	34.0	5.6	5.6	8.61	349.5	1.235	2.650	53.4	46.5	6.9
25-50	32.9	5.8	7.2	9.91	402.4	1.240	2.655	53.4	48.6	4.8
50-75	31.7	8.3	12.2	10.41	422.6	1.250	2.655	53.1	49.0	4.1
75-100	31.0	7.6	12.9	10.64	432.0	1.260	2.655	52.5	52.5	0.0

\* Multiplied by 100.

† Of dry soil.

changes occur in the tested soil samples from the northern part of the Jordan Valley:

Na/Ca ratio of replaceable cations .....	increases
Internal surface .....	increases
Capillary rise .....	increases
Downward flow of water .....	increases
Noncapillary porosity .....	disappears

It may therefore be presumed that the Na/Ca ratio of the replaceable cations is the decisive factor in the change of soil-water properties and in the formation of the internal soil structure.

This suggestion requires confirmation from the point of view of physics and colloidal chemistry. To obtain this, the relation between the properties observed should be established by the use of equations accepted in physics for molecular phenomena, and the general colloid-chemical aspects of the subject should be studied.

A general scheme showing the varying internal soil-structure properties in correlation with the variations in replaceable cations may thus be constructed and later verified.

**INTERNAL SURFACE, NONCAPILLARY POROSITY, AND DOWNWARD MOVEMENT OF  
WATER IN SOIL IN RELATION TO CHANGE OF Na/Ca RATIO OF  
REPLACEABLE CATIONS**

The last column in table 2 demonstrates the gradual disappearance of the non-capillary porosity with increasing depth. As the Na/Ca ratio of replaceable cations increases with depth, it is reasonable to assume that the decrease in percentage of noncapillary porosity is connected with its transformation into the capillary porosity under the influence of the increased Na/Ca ratio of replaceable cations.

Let us now assume that the bulk of the soil is composed of definite capillary tubes, the number of which increases with the transformation of noncapillary porosity volume into capillary tubes. This hypothesis will be justified only if the variations of the internal surface calculated from the observed durations of water flow through the soil columns coincide with the variations of the internal surface measured according to Mitscherlich.

The internal surface ( $S_n$ ) for the unit of length and the same section will then be the function of the number of capillary tubes ( $n$ ).

$$\frac{S_{n_1}}{S_{n_2}} = \frac{n_1 2\pi r}{n_2 2\pi r} = \frac{n_1 D}{n_2 D} \quad (1)$$

where  $D$  is the diameter of the average capillary tube.

From equation (1) it follows that the relation of the surfaces may be replaced by the relation of the average tube diameter multiplied by the number of the suggested capillary tubes.

For the water flow through the cylindrical tube, Poiseulle (1, p. 529) formulated the following classical equation:

$$Q = \frac{KPD^4}{L} T \quad (2)$$

in which  $Q$  = quantity of water,  $P$  = pressure,  $D$  = diameter of the tube,  $L$  = length of the tube,  $T$  = duration of the water flow, and  $K$  = coefficient. For the same quantity of water ( $Q$ ) and constant height of the water column ( $L$ ), constant  $P$ , and variable  $D$ , equation (2) becomes

$$D = K_c \frac{1}{\sqrt[4]{T}} \quad (3)$$

where  $K_e = \sqrt[4]{\frac{QL}{KP}}$  has a constant value. For the flow of water through a group of similar capillary tubes, we may accept  $D_n = nD$ , where  $n$  is the number of tubes in the group. If we now substitute in equation (3) the durations of the water flow for the underlying layers of soil (table 1), we obtain figures which, according to formula (1), represent the relative internal surface areas for different layers of soil.

The identity, within the limits of error, between the calculated internal surface area and the measured internal surface by Mitscherlich's method (table 3) justifies the hypothesis that the investigated soil is composed of uniform capillary tubes and that the increase of Na/Ca ratio of replaceable cations transforms the noncapillary porosity into capillary tubes of the same kind.

TABLE 3

*Ratio between internal surfaces, calculated and observed, in gray soil of the northern part of the Jordan Valley*

DEPTH cm.	$D_n$ FROM EQUATION (3)	INTERNAL SURFACES*		INTERNAL SURFACE 1 GM. DRY SOIL	
		Calculated from equation (3)	After Mitscherlich (table 2)	Calculated from equation (3)	After Mitscherlich- Rodewald
0-25	$\frac{1}{\sqrt[4]{138}} K_e$	1.0	1.0	349.5	349.5
25-50	$\frac{1}{\sqrt[4]{72}} K_e$	1.17	1.15	408.4	402.4
50-75	$\frac{1}{\sqrt[4]{60}} K_e$	1.25	1.21	429.9	422.5
75-100	$\frac{1}{\sqrt[4]{50}} K_e$	1.29	1.24	450.8	432.5

\* Internal surface of the first soil-layer accepted as = 1.0.

The differences between calculated and observed internal surface areas are further explained by the diminution in the diameters of equivalent capillary tubes as influenced by replaceable cations.

#### CAPILLARY RISE OF WATER IN SOIL IN RELATION TO HYDRATION OF REPLACEABLE CATIONS OF "SOIL CAPILLARY TUBE"

The equation for the height to which water ascends in a vertical capillary tube is

$$h = \frac{2\alpha}{gr} \cos \theta \quad (4)$$

where  $h$  represents height of meniscus above water level;  $r$ , radius of the tube;  $\alpha$ , surface tension between water and air;  $\theta$ , angle of wetting;  $g$ , acceleration of

gravity. In complete wetting,  $\theta = 0$ , and  $\cos \theta = 1$ . Substituting the numerical values in equation (4), we have

$$r = \frac{0.15}{h} \quad (5)$$

The admission of the hypothesis that soil structure is composed of capillary tubes permits the application of equation (4) to the upward rise of water in soil (table 4). It follows from this equation that capillary rise either increases together with the growth of the surface tension of the liquid phase or changes inversely to the equivalent radius of the soil capillary tube. In a previous paper (8) it was shown that the soil solution reflects the composition of the exchangeable bases in the "adsorbing soil complex" and that an increase in the amount of any adsorbed cation in the soil causes an increase in the amount of the same cation in the soil solution. Moreover, it was shown that Na is more completely hydrolyzed than Ca (8, pp. 60-61). It may therefore be assumed that if increased quantities of adsorbed K and Na cations are present in the soil, the soil solution also contains

TABLE 4  
*Capillary rise of water in gray soil of the northern part of the Jordan Valley*

DEPTH cm.	CAPILLARY RISE OF WATER									
	1 day cm.	2 days cm.	3 days cm.	4 days cm.	5 days cm.	6 days cm.	7 days cm.	8 days cm.	9 days cm.	10 days cm.
0-25	19.3	22.8	24.8	26.5	28.0	29.2	30.2	31.2	32.0	32.8
25-50	21.6	25.2	27.0	28.7	29.7	30.8	31.8	32.7	33.5	34.3
50-75	21.7	25.4	27.4	29.3	30.5	31.8	33.1	34.1	35.0	35.8
75-100	22.4	26.2	28.4	29.6	31.4	32.3	33.8	34.8	35.8	36.6

increasing quantities of the same cations; and if the soil is treated with pure water, KOH and NaOH will be found in solution. The higher surface tension of water solutions of KOH and NaOH in comparison with that of pure water has already been established (2, p. 83). If we grant the effect, due to their higher surface tension, of KOH and NaOH formed in the soil solution upon the capillary rise of water in the soil, the share of these hydroxides in the phenomenon cannot be great. In explaining the causes of the gradual rise of capillary water in the underlying layers of the soil, however, this minor action of the growing quantities of absorbed monovalent cations cannot be excluded.

Two factors contribute primarily to the formation of soil porosity, which is responsible for the state of soil moisture and aeration: the sand fraction, composed of weathered rock fragments, and the colloidal clay fraction. The sand fraction (the skeleton of the soil) maintains constant form and volume, undergoing only slight alterations. The porosity of this portion of the soil remains unchanged. The colloidal clay, on the other hand, influenced by external factors (water, electrolytes, and temperature) undergoes a fundamental change in its physico-

chemical properties and this, in turn, affects the volume and form of the soil porosity. The chemical composition of the clay, under these circumstances, remains more or less constant. According to Wiegner (14, p. 15), the nucleus of the colloidal clay fraction of soil is composed of kaolin, which has a fine, porous orientated structure. The cation exchange in soil possesses, therefore, an extramicellar character and takes place on the micelle surface. The degree of hydration of the micelle surface varies according to the kind of "replaceable cation," and the shape of the soil structure is modified accordingly. In the process of hydration two types of structure transformations are probable: first, the "soil caverns"<sup>1</sup> are converted into capillary tubes; second, the diameters of the original capillary soil tubes change. The first probability is based on the observations which show that the internal-surface figures calculated from the downward flow in soil coincide with the figures obtained by the method of Mitscherlich. The

TABLE 5

*Exchangeable cations, hydration, and radii of soil capillary tubes, in gray soil from the northern part of the Jordan Valley*

DEPTH cm.	EXCHANGEABLE CATIONS IN 100 GM. DRY SOIL				HYDRATION OF EXCHANGEABLE CATIONS*			$r$ OF HYPOTHETICAL CAPILLARY TUBE†	CURVATURE $\frac{1}{r} \cdot 100$	HYDRA- TION/CUR- VATURE RATIO
	K	Na	Ca	Mg	Mono- valent	Bivalent	Total			
	m.e.	m.e.	m.e.	m.e.	mgm.	mgm.	mgm.			
0-25	0.41	1.50	26.6	5.47	106	481	587	46	2.17	270
25-50	0.12	1.78	24.8	6.23	120	472	592	44	2.27	261
50-75	0.0	2.62	21.5	7.57	173	453	626	42	2.38	263
75-100	0.0	2.34	18.3	10.31	156	462	618	41	2.44	253

\* Hydration values, molecules H<sub>2</sub>O to 1 cation-equivalent—Na<sup>+</sup>, 66; K<sup>+</sup>, 16;  $\frac{\text{Mg}^{++}}{2}$ , 20;

$\frac{\text{Ca}^{++}}{2}$ , 14 (2, p. 81).

† Calculated from last column of table 4, according to equation (6).

second may be supported by the possibility that the degree of hydration of the colloidal clay in soil, induced by replaceable cations, corresponds to the alterations in the equivalent radius, calculated for the average soil capillary tube.

Molecules of H<sub>2</sub>O bound with the replaceable cations and values  $(1/r) \cdot 100$ , where  $r$  is calculated from equation (5) and represents the hypothetical equivalent radius of the soil capillary tube, are recorded in table 5. The inverse radius,  $\frac{1}{r}$ , represents the curvature of the tube.

The figures for hydration/curvature ratio in the four layers of soil, given in the last column of table 5, demonstrate parallel changes of curvatures ( $1/r$ ) of the equivalent soil capillary tubes and of the corresponding degrees of hydration of replaceable cations. Thus the interrelation between replaceable cations and equivalent radius of soil tubes is confirmed.

<sup>1</sup> "Soil caverns" are large soil-air spaces of noncapillary form.

In the formation of soil porosity, the role of the sand fraction decreases in importance in the presence of colloidal clay. Even when only a small quantity of clay is present in the soil, the fine clay particles cover the rock fragments with a thin colloidal film, and direct contact of sand, water, and air is prevented. For soils with a high proportion of clay, the soil-water conditions are the same as if the soil were composed only of clay.

Interpretation of the data on soils with a low content of monovalent replaceable cations corroborates the close connection between soil capillary structure and replaceable cations.

TABLE 6

*Replaceable cations, hygroscopicity, internal surface, specific gravity, and porosity in heavy loamy soil from the coastal plain*

DEPTH cm.	TOTAL CATIONS IN 100 GM. DRY SOIL m.e.	MONOVA- LENT CATIONS, PERCENTAGE OF TOTAL	Na/Ca RATIO*	HYGRO- SCOPICITY per cent†	INTERNAL SURFACE 1 GM. DRY SOIL sq. m.	APPARENT SP. GR.	REAL SP. GR.	TOTAL POROSITY per cent	CAPIL- LARY POROSITY per cent
0-25	66.7	11.4	16.0	14.34	582.1	1.290	2.71	52.4	53.0
25-50	59.7	7.6	10.2	15.15	615.2	1.290	2.74	53.0	53.5
50-75	59.9	13.4	21.0	16.02	650.3	1.290	2.76	53.5	53.9
75-100	64.2	19.0	35.6	16.48	668.9	1.310	2.78	53.0	54.0

\* Multiplied by 100.

† Of dry soil.

TABLE 7  
*Downward movement of water in loamy soil from coastal plain*

TIME REQUIRED TO FILL 50-CC. FLASK WITH DRAINAGE WATER

cm.	First portion of water	Second portion of water	Third portion of water
0-25	3 hours 55 minutes	5 hours	6 hours
25-50	6 hours 40 minutes	10 hours 30 minutes	17 hours 30 minutes
50-75	9 hours	15 hours 20 minutes	29 hours
75-100	169 hours	235 hours	306 hours

#### SOIL-WATER PROPERTIES OF SOILS WITH A HIGH CONTENT OF MONOVALENT REPLACEABLE CATIONS AND INCREASING Na/Ca RATIOS

For the study of soil-water properties of soils with a high content of monovalent cations, samples of heavy loamy soils from the coastal plain (9) were selected. These soils are distinguished by marked decomposition of mineral material without the transport of sesquioxides. They contain large quantities of monovalent replaceable cations (table 6), which increase with depth, and they possess chemically homogeneous soil profiles.

The figures for the general properties of the soil samples taken from different

layers of the loamy coastal plain soil and the values for the replaceable cations are recorded in table 6. The data on the soil-water properties are given in table 7 and figure 2. From these data it is obvious that the following changes occur in properties with increasing depth:

Na/Ca ratio of replaceable cations.....	increases
Internal surface.....	increases
Capillary rise.....	decreases
Downward flow of water.....	decreases
Noncapillary porosity.....	always absent

The disappearance of the noncapillary porosity and the deterioration of the water-conductivity system (decrease of capillary rise and downward flow) can be

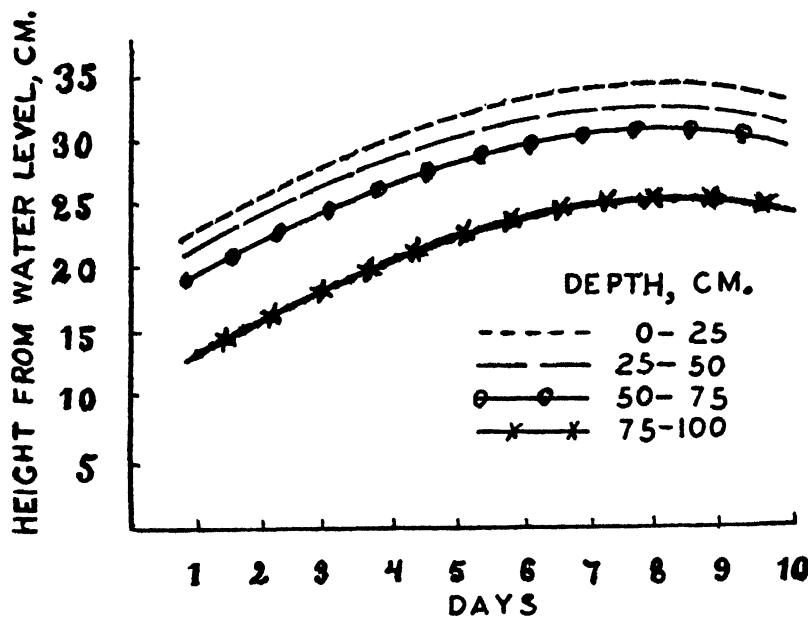


FIG. 2. CAPILLARY RISE OF MOISTURE IN LOAMY SOILS FROM THE COASTAL PLAIN

explained by the onset of a process of hydration, which creates a condition of partly closed foramen-apertures of the capillary soil tubes, and results in the colloidal clay's commencing its transformation into sol.

The results of our study of the relations between the Na/Ca ratio of replaceable cations, on the one hand, and soil-water properties and physical structure, on the other, are roughly represented in figure 3. The horizontal axis represents the Na/Ca ratio of replaceable cations; the vertical one, the capillary rise. Curve ABCD shows an initial increase of the capillary rise dependent on the increase of the Na/Ca ratio. Subsequently the curve rises to a maximum and then begins to descend. Further increase in the Na/Ca ratio is accompanied by a decline of the capillary rise. The growth of the internal surface simultaneously with the

increase in the Na/Ca ratio is shown by the straight line *EF*. The other important phenomenon—the gradual disappearance of noncapillary porosity in the same direction—is represented by the curve *GH*.

Figure 3 shows that the initial enrichment of the soil colloidal clay by monovalent replaceable cations results in an increase of capillary rise and downward water flow. These circumstances are propitious for plant nutrition and favorable to the exchange of nutritive elements between soil and plant. On the other hand, the soil treatment by which the amount of monovalent exchangeable cations and the Na/Ca ratio attain a high value, instead of ameliorating the situation, causes a sharp decline of the water-conductivity system in soil and a general deterioration in its physicochemical condition.

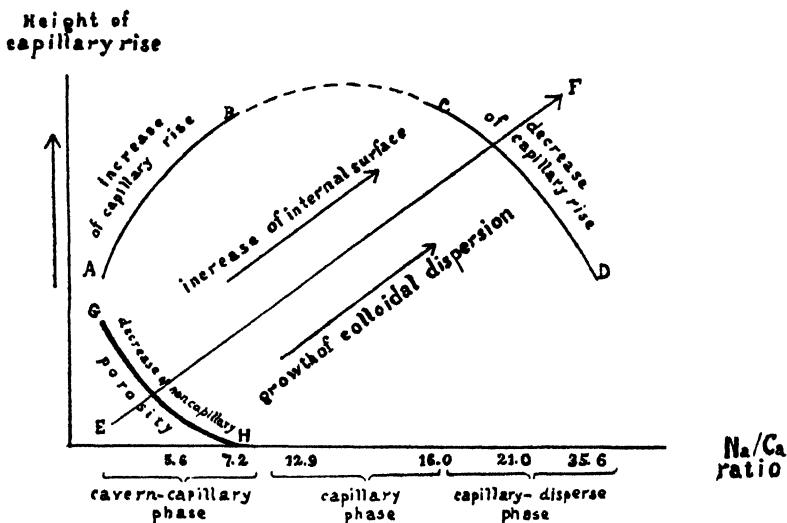


FIG. 3. SCHEME REPRESENTING THE INFLUENCE OF Na/Ca RATIO OF REPLACEABLE CATIONS ON THE CAPILLARY RISE OF WATER IN SOIL, ITS POROSITY, AND INTERNAL SURFACE

#### DISCUSSION

Interpretations have been given by some authors (5) of the rules regulating soil-water relations as induced by the geometrical form of soil pore-space. A spherical shape was ascribed to the "ideal" soil particles, and the size and form of the soil pores were attributed to the wetting and gradual filling of the cells with water. According to this interpretation the size and form of the spores in dry and wet soil are determined solely by the rules of physics, while the material of which the soil is built remains inert and chemically neutral during the process of soil-structure formation. This view of the soil capillary tubes as a chain composed of tetrahedral and rhomboidal cells is absolutely theoretical and cannot be applied even to fine sand formed from broken rock, and certainly not to soil that contains colloidal clay, the properties of which determine the nature of soil.

Colloidal clay, which is present in soil and covers the chemically inert rock

particles with a fine film, serves as a medium for the construction of soil pores and capillaries. The swelling process, which depends on the rate of penetration of water molecules in the clay, increases the clay pressure and contracts the capillary tubes of the soil. The parallel course of the curves for capillary water rise supports this point of view, suggesting the gradual swelling of the clay in relation to some constant agent.

Replaceable cations with a different coefficient of hydration subject the swelling of colloidal clay to the effect of Hofmeister's series of cations and govern the formation process of soil capillaries.

Three subsequent phases in the development of soil structure under the influence of replaceable cations are represented in figure 3. The first phase, where the transformation of "soil caverns" in capillary tubes begins, was termed "cavern-capillary." In the second phase—the capillary—the soil is composed of capillary tubes. The further increase in the amount of monovalent replaceable cations as well as in the Na/Ca ratio creates the last "capillary-disperse" phase, which comprises the beginning of peptization of colloidal clay.

In the medium composed of rock fragments, covered with a wet film of colloidal clay, there is no room for the formation of rhomboidal and tetrahedral cells. The swelling of colloidal clay produces on its surface an elastic film, which has a tendency to contract and thus form curved shapes; in approaching equilibrium it creates cavities more or less similar to the capillary tube. The capillary form of soil cavities is therefore a result of the equilibrium attained through the interference of surface forces in a precise colloidal system. The change of the surface tension of a colloidal film may cause a decrease in the equivalent capillary tube radius, or the fall of the "soil caverns" into some capillary tubes. The latter phenomenon corresponds to the breaking of a wave and its transformation into an agglomeration of tiny drops. It can be explained by the presence of the surface tension of the first and the second kind, between which equilibrium exists.

Ostwald (12), in explaining the conditions of colloidal dispersion, writes:

So well does the presence of "expansive tension" prevent the shortening of the total volume, that the "contractive tension" can be met only by breaking the volume into small pieces. In such a case splitting meets both demands: on the one hand by increasing the total surface the "expansive tension" is satisfied, while on the other hand the reduction of the surface of the separate parts satisfies the "contractive tension."

The swelling of colloidal clay represents the transition of gel to sol, accompanied by the absorption of water. The process is part of the phenomenon in which "expansive tension" is satisfied (by the increase of the total surface). The tendency of "contractive tension," the second factor, is satisfied by the transformation of "soil caverns" into capillaries or by the decrease of the capillary radius under the influence of swelling. In the soil conducting water, swollen and dispersed colloidal clay forms an elastic film, on which, according to Gibbs (3), "capillary active" substances that are always present in soil also accumulate. Under the influence of this surface tension the film builds pores and cavities which merge to form capillaries, and these serve to convey the water and water-soluble substances.

The facts established and the observations made permit us to interpret the soil-water conductivity system in the soils investigated as composed of capillary tubes formed of colloidal clay under the influence of the "replaceable cations."

#### SUMMARY AND CONCLUSIONS

The study of the soil-water properties of mineral soils in relation to the Na/Ca ratio of replaceable cations reveals a close connection between these properties and the Na/Ca ratio.

In soils with a low content of replaceable monovalent cations, the increase in Na/Ca ratio induces a gradual increase of the capillary rise as well as of the downward water flow.

In soils with a high content of replaceable monovalent cations, the increase in Na/Ca ratio induces a gradual lowering of the capillary rise as well as of the downward water flow.

Internal surface, measured according to Mitscherlich, increases continuously in accordance with the Na/Ca ratio of replaceable cations.

Noncapillary porosity gradually decreases with the increasing Na/Ca ratio of replaceable cations and disappears at a definite point.

It is suggested: first, that these soil-water properties are produced by the change in number and diameter of the "equivalent capillary tubes" in the soil; and second, that these variations of diameter are due to different degrees of swelling of the soil colloidal clay.

These interpretations were verified by calculation and observation as follows:

The figures calculated by the Poiseulle formula for the internal surface in the samples with changing amounts of replaceable cations were found to agree with the figures observed according to Mitscherlich.

The line representing the ratio of the total hydration of replaceable cations to the calculated curvatures  $\left(\frac{1}{r} \cdot 100\right)$  of the capillary soil tube is horizontal and demonstrates a constant correlation in the investigated soils between the state of swelling of soil clay induced by replaceable cations and the equivalent soil tube radius.

The gradual changes in the soil structure connected with the amelioration of the water conductivity system in soils with a low Na/Ca ratio of replaceable cations and the deterioration of this system in soils with a high Na/Ca ratio are explained by the swelling of soil colloidal clay induced by replaceable cations.

The soil structure which determines soil-water properties is a state of equilibrium in colloidal soil clay, created by the influence of two opposite forces of surface tension.

#### REFERENCES

- (1) CHWOLSON, O. 1908 *Cours de Physique*, vol. 1. Paris.
- (2) FREUNDLICH, H. 1922 *Kapillarchemie*. Leipzig.
- (3) GIBBS, J. W. 1928 *Thermodynamics*. [In Collected works, vol. 1, (edited by W. R. Langley and R. G. Van Name). Longman's Green & Co., New York.]
- (4) HAGER, G. 1924 *Die Methoden zur Untersuchung der Bodenkolloide und ihrer*

- Eigenschaften. (In E. Abderhalden's Handbuch der Biologischen Arbeitsmethoden. Urban u. Schwartzenberg, Berlin und Wein.)
- (5) HAINES, W. B. 1927 Studies in the physical properties of soils: IV. A further contribution to the theory of capillary phenomena in soil. *Jour. Agr. Sci.* 17: 264-290.
- (6) HISSINK, D. J. 1922 Beitrag zur Kenntnis der Absorptionsvorgänge im Boden. *Internat. Mitt. Bodenk.* 12: 81-171.
- (7) KEEN, B. A. 1931 The Physical Properties of Soil. London.
- (8) MENCHIKOVSKY, F., AND RAVIKOVITCH, S. 1929 The influence of the replaceable bases on the soil solution in mineralized soils. *Soil Sci.* 27: 49-68.
- (9) MENCHIKOVSKY, F. 1938 The genesis of some loamy soils of Palestine, especially regarding their colloidal part. *Soil Res.* 6: 1-20.
- (10) MENCHIKOVSKY, F., AND PUFFELES, M. 1938 The relation of exchangeable cations to the "active" aluminum in soil. *Soil Sci.* 45: 25-28.
- (11) MITSCHERLICH, E. A. 1920 Bodenkunde. Berlin.
- (12) OSTWALD, W. 1921 Grundriss der Kolloidchemie. Berlin.
- (13) WAGELER, P. 1932 Die Kationen und Wasserhaushalt der Mineralboden. Berlin.
- (14) WIEGNER, G. 1936 Ionenumtausch und Struktur. *Trans. Third Internat. Cong. Soil Sci.* (1935) 3: 5-28.



# THE WATER TABLE, EQUIPOTENTIALS, AND STREAMLINES IN DRAINED LAND: IV. DRAINAGE OF FOREIGN WATER

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The simplest problem arising in connection with foreign water may be stated as follows: Given an impermeable bed, uniformly sloping at an angle  $\theta$  with the horizontal, upon which rests a layer of soil of uniform permeability  $P_u$ , as defined in part I (2), and down which flows ground water at a steady rate  $dQ/dt$  per unit width measured at right angles to the direction of fall, what is the nature of the potential field, which determines the streamlines and the location of the water table, and how is it affected by a drain installed so as to intercept the ground-water flow? It is assumed that the land surface extends to a great distance (effectively infinity) in all directions from any point at which the stream picture is being considered. In the absence of the drain, we have the well-known result that, if we consider a vertical soil section containing the direction of fall, the flow net consists of a rectangular array of equipotentials and streamlines, the former being perpendicular to, and the latter parallel with, the impermeable bed. The thickness  $T$  of the zone of flow, measured perpendicular to the bed, is given by the equation

$$dQ/dt = P_u T g \rho \sin \theta \quad (1)$$

in which  $g$  and  $\rho$  have their usual significance, the gravitational acceleration and the density of the flowing water respectively.

The effect of a foreign-water drain<sup>1</sup> was elucidated by the theoretical work of Hopf and Trefftz (5) in 1921, a solution for certain restricted cases being obtained by a neat application of the Schwarz-Christoffel transformation. The solution was limited to the case of an open ditch running across the slope, the shape of the ditch not being preassigned but emerging in the solution as a consequence of adopting arbitrary values for certain parameters. The surface of seepage on the uphill bank of the ditch and the capillary fringe above the water table both had to be ignored. The method of electric analogues is not subject to these limitations, and the purpose of this paper is to present solutions obtained by this method for various cases of buried pipe drains and open ditches, laid at different depths in soil resting on beds of different gradients.

The method of electric analogues as applied to drainage problems has already been described in detail (2, 3, 4), and it will suffice here to discuss the conditions to be satisfied in our present particular problem. Figure 1 shows a cross sec-

<sup>1</sup>Drains of the type discussed in this paper are often referred to simply as "interceptor drains." Since all drains are interceptor drains in that they intercept and run off water which otherwise would run elsewhere and constitute a nuisance, some such name as that used here is preferable.

tion of the ground-water zone with an impermeable bed sloping at an angle  $\theta$  and with an open ditch running across the slope to intercept the flow. It will be assumed that the soil pores are very uniform in size, so that the capillary fringe is well defined (3), with its upper boundary forming the interface between the lower zone of saturation permeability and the upper zone of negligible permeability. The distance on the uphill side at which the drain exerts an appreciable influence is limited, so that at remote distances the flow approaches asymptotically the undisturbed state described by equation (1). On the down-hill side at distances beyond the local disturbance due to the drain, an equation similar to (1) must hold, namely,

$$(dQ/dt)(1 - a) = P_w \tau g \rho \sin \theta \quad (2)$$

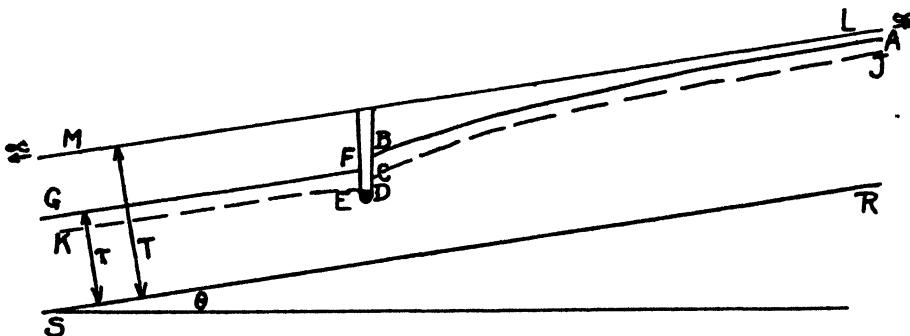


FIG. 1. EFFECT OF A DRAIN ACROSS THE SLOPE, INTERCEPTING FOREIGN WATER

The source is infinitely distant in the *L* direction, the sink in the *M* direction.

*ML* is the boundary of the capillary fringe before the ditch is dug.

*ABFG* is the boundary after the ditch is dug.

*JCEK* is the water table after the ditch is dug.

*CD* is the surface of seepage on the uphill bank of the ditch.

*DE* is the surface of the water in the ditch.

*RS* is the sloping impermeable bed.

where  $a$  is the fraction of the incident flow  $dQ/dt$  removed by the drain and  $\tau$  is the reduced thickness of the flow zone. In the case of a covered pipe drain, the only differences from the case described are that the soil and the capillary fringe boundary are continuous above the drain and that there is no surface of seepage, assuming that the drain is just full to the roof with water.

The nature of the appropriate analogue is tolerably obvious. It consists of a sheet conductor cut in the form of a strip, with current led in at the "uphill" end by an electrode cemented across the whole width, since little error is made by assuming the equipotentials at reasonable distances from the ditch to be straight lines perpendicular to the strip edges. The exit current is divided between a similar electrode at the "downhill" end and an electrode simulating the drain in dimensions and location. The scale having been decided upon, the strip must be cut to the streamlines *AB* and *FG* and the electrode potentials adjusted, all by trial and error, until two conditions are simultaneously satis-

fied. The first is that the electric potential,  $V$ , at the "fringe boundary" must satisfy the equation (3)

$$V = Ah - C \quad (3)$$

where  $V$  is measured relative to the drain electrode;  $A$  is a constant relating  $V$  at the water-table analogue to the hydraulic potential,  $\phi$ , at the corresponding point in the soil,  $\phi$  being defined as in part I (2);  $h$  is the height of the point on the "fringe boundary" above the level of the drain-water surface; and  $C$  is a constant representing the pressure,  $p_e$ , at which the previously saturated soil loses its water content. The second condition is that the conductor must tend

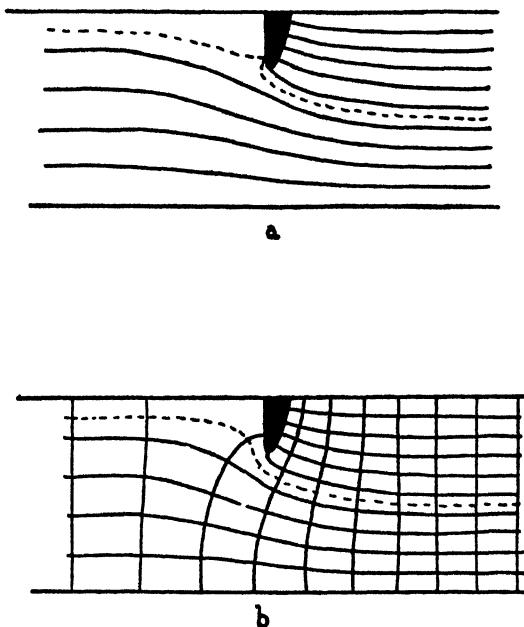


FIG. 2. a. Streamlines calculated from the theory of Hopf and Trefftz, with a bed gradient of 1 in 300 and half the flow intercepted, the lower 45 per cent not touching the ditch at all. The dotted line is the 45 per cent streamline. b. The same case treated by the method of electric analogues.

to a parallel strip of width  $W$  on the "uphill" side and  $w$  on the downhill side, where  $W$  represents to scale the thickness of the undisturbed flow zone and  $w$  is given by

$$w/W = 1 - i_D/i \quad (4)$$

where  $i$  is the total current fed in at the "uphill" electrode and  $i_D$  is that led out by the drain electrode. Equation (4) is analogous to that resulting from a combination of (1) and (2), namely

$$\tau/T = 1 - a \quad (5)$$

Where a surface of seepage is found to develop, it must be represented by an

electrode cemented to the conductor along the cut representing the uphill ditch bank, and having maintained along it a potential fall such that (3)

$$V = Ah \quad (6)$$

This equation also governs the analogue of the water table, which may be traced, together with the complete flow network, upon the satisfactory completion of the electric analogue.

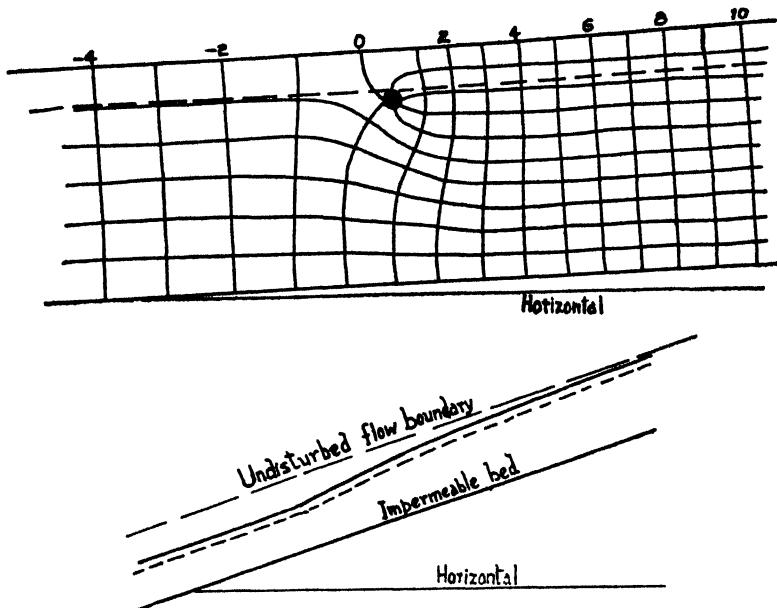


FIG. 3. INTERCEPTION BY A 10-CM. DRAIN LAID AT A DEPTH OF 1 METER IN A FLOW ZONE. ORIGINALLY 2 METERS THICK, THE BED GRADIENT BEING 1 IN 30

The broken line is the water table. The upper figure is the detail near the drain; the lower figure represents a greater extent to smaller scale. In the lower figure the vertical scale is 10 times the horizontal scale, and one fifth the scale of the upper figure. The equipotentials are labeled in units of 1,000 ergs per cubic centimeter.

#### EXPERIMENTAL PROCEDURE AND RESULTS

There is little of special interest to report as to the construction of the analogues. In the earlier experiments the sheets were cut to considerable distances on each side of the drain electrode, but in later work the sheet was confined to the region of local disturbance due to the drain, where the detailed tracing of the flow network is of interest, the shape of the flow boundary at remoter distances being calculated by a numerical process of trial and error. The undisturbed flow width was taken as 10 cm. (figs. 3 to 8) and has been interpreted, for the sake of presenting specific field cases, as representing a ground-water flow zone 2 meters thick. Since the stream picture is the same on any scale, any other interpretation requires only the relabeling of the equipotentials.

The first experiment, carried out as a check on the method, was a reproduc-

tion of the case presented in detail by Hopf and Trefftz (5), with  $\sin \theta$  equal to  $1/300$ , an open ditch with cross section as calculated by them, and with both capillary fringe and surface of seepage ignored. Figure 2a is the theoretical stream picture, and was recalculated because that published by Hopf and Trefftz was in insufficient detail, and in particular because the streamline which just touches the ditch (the only intermediate streamline presented) seems to have been drawn in freely and with considerable error. Figure 2b is the flow net obtained by the electric analogue. In figure 2a the streamlines are calculated directly, whereas in figure 2b they are drawn in as orthogonals to the experimentally derived equipotentials, which are therefore also included in the latter figure. The agreement is satisfactory.

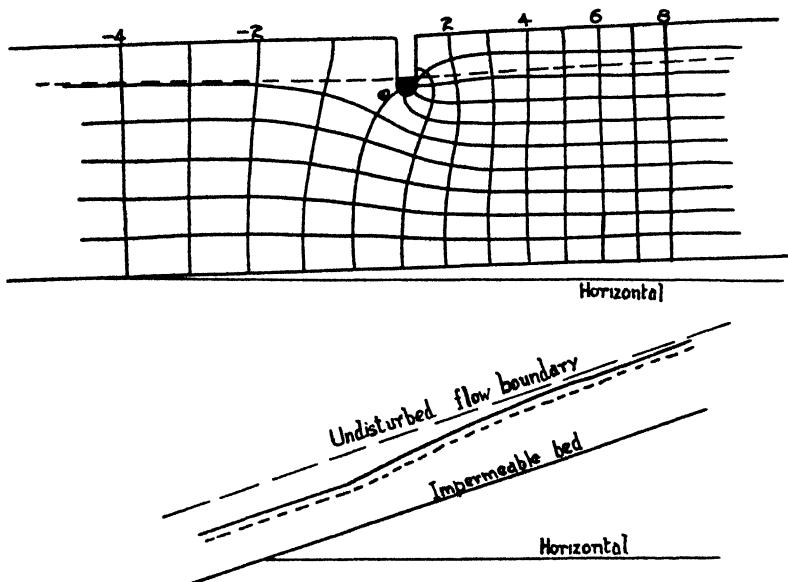


FIG. 4. SAME AS FIGURE 3, BUT WITH AN OPEN DITCH INSTEAD OF A COVERED PIPE

In the remaining figures, the drains are either, to the scale mentioned above, covered pipes of 10 cm. diameter (about 4 inches) or open ditches of equivalent conducting section. The other circumstances are adequately indicated in the respective legends. In constructing the analogues, no attempt was made to represent any particular soil, and for simplicity a thickness of capillary fringe, rather than a value of  $C$  in equation (3), was assumed.

#### DISCUSSION

A foreign water drain is installed primarily to protect the land on the down-hill side, and the figures show that, for this purpose, the efficacy of a buried pipe drain as compared with an open ditch depends on the slope of the bed. On a slope as steep as 1 in 30, both types of drain effectively limit the height of the water table to a distance above the bed equal to that of the surface of the water

in the drain, that is, both are completely effective. At the worst, with the pipe laid directly on the bed, only 1 per cent more of the total flux passes the drain than in the case of an open ditch dug to the same depth. Although the experiment was carried out, this latter case is not illustrated, since it is obvious that such a ditch must completely intercept the flow on the uphill side and act as a source for flow on the downhill side with calculable consequences. When the bed slope increases to 1 in 3, control by pipe drain is imperfect, and the efficacy of an open ditch may or may not be complete. When the pipe roof (or the surface of water in a ditch) is 1 meter from the bed, that is, in the middle of the

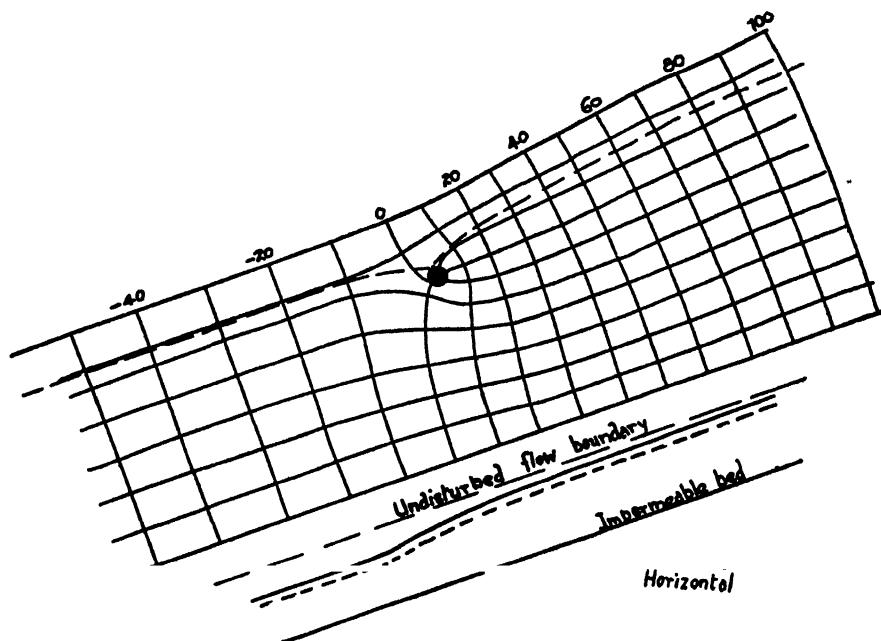


FIG. 5. SAME AS FIGURE 3, BUT WITH A BED GRADIENT OF 1 IN 3  
In the lower figure the scale is not distorted, and is one fifth that of the upper figure.

*undisturbed* flow zone, with the floor of the drain or ditch 10 cm. deeper, the water table on the downhill side is 110 cm. above the bed for both types of drain, instead of the 100 cm. corresponding to perfect control. Allowing for the assumed 20 cm. thickness of capillary fringe, 65 per cent of the total flux is passed on to the downhill side instead of the 60 per cent, for perfect control, achieved at the lower slope.<sup>2</sup> With the floor of the drain on the bed, the covered

<sup>2</sup> The fact that perfect control, with a drain in the middle of the undisturbed flow thickness, corresponds to a diversion by the drain of only 40 per cent of the total flow, and not 50 per cent, is a reflection of the influence of the capillary fringe, which is assumed to have a thickness of 20 cm. The water table is effectively brought down to drain level, that is, from 180 cm. above the bed to 100 cm., but the capillary fringe boundary is reduced from 200 cm. above the bed to only 120 cm. This effect of additional conductance in the capillary fringe in cases of sideways flow has been discussed in Part II (3, p. 323).

pipe passes on 25 per cent of the total flow instead of the 15 per cent, corresponding to perfect control, to be expected of an open ditch dug to this level.

On the uphill side of the drain, the influence on the flow boundary and water table extends for a distance which is greater the more gradual is the gradient of the bed. In fact, if we compare figures 4 and 6 and the published curve of Hopf and Trefftz for a gradient of 1 in 300, we find that the distance from the drain at which a given degree of control is exerted is roughly inversely proportional to the gradient of the bed. For a reduction in the thickness of the flow zone equal to 50 per cent of the maximum, the distance from the drain is approximately 3, 30, and 300 meters for slopes of 1 in 3, 1 in 30, and 1 in 300 re-

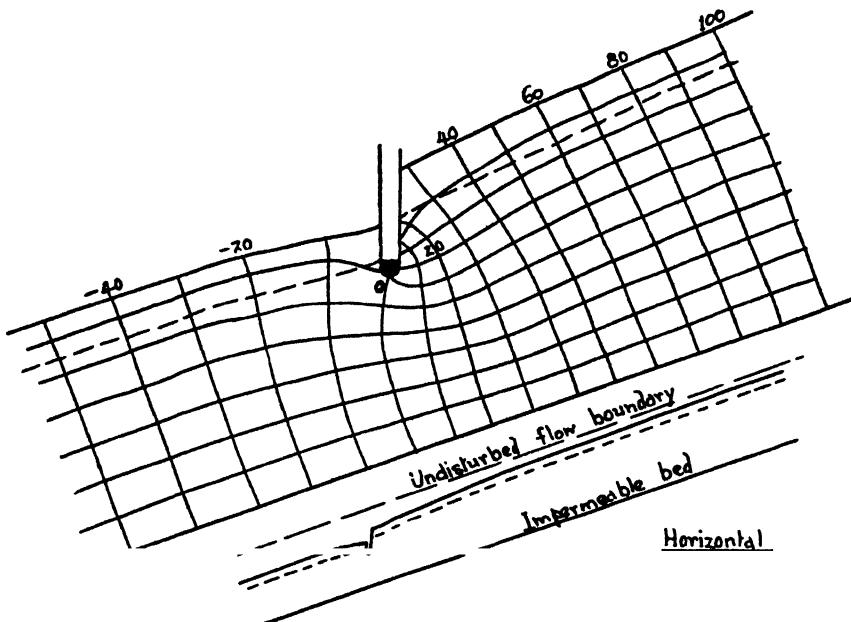


FIG. 6. SAME AS FIGURE 5, BUT WITH AN OPEN DITCH INSTEAD OF A COVERED PIPE

spectively. It may be shown that this is to be expected. Suppose, for example, we have two soils of equal permeability and thickness of undisturbed flow, with similar drains at the same depth, but for one of which the bed slope,  $\sin \theta_n$ , is  $n$  times that of the other,  $\sin \theta_1$ . Then, by an application of equation (1), we have

$$(dQ/dt)_n / (dQ/dt)_1 = \sin \theta_n / \sin \theta_1 = n \quad (7)$$

where subscripts attached to any symbol indicate the case to which that symbol refers. Now, except in the vicinity of the drain, the equipotentials are approximately planes normal to the direction of slope of the bed, and the potential gradient is therefore expressed by  $d\phi/dl$ , where  $l$  is distance from the drain measured along the bed, not along the horizontal. If we compare potential gradients in the two cases at points on the uphill side where the thickness,  $z_n$ ,

of the one flow zone is the same as that,  $z_1$ , of the other, equation (7) implies the relation

$$(d\phi/dl)_n = n(d\phi/dl)_1 \quad (8)$$

for

$$z_n = z_1$$

At the capillary fringe boundary, which also bounds the flow zone, the hydraulic potential  $\phi$  is given by (3)

$$\phi = gph + p_0 \quad (9)$$

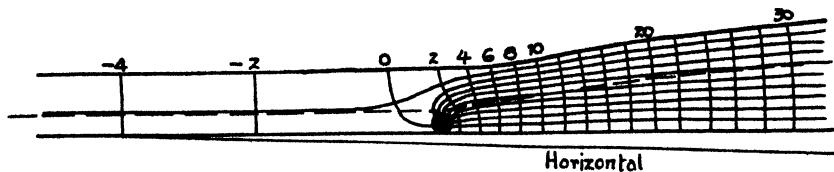


FIG. 7. SAME AS FIGURE 3, BUT WITH THE DRAIN LAID ON THE BED

where  $h$  is vertical height above the datum level, namely, that of the surface of the water in the drain. If this latter surface is distant  $z_D$  above the bed, then  $h$  at a point on the boundary of the flow zone is given by

$$h = l \sin \theta + (z - z_D) \cos \theta \quad (10)$$

and by substituting this value in (9), differentiating, and supplying subscripts appropriate to our two special cases under discussion, we get the pair of equations

$$\begin{aligned} (d\phi/dl)_n &= g\rho \{ \sin \theta_n + (dz/dl)_n \cos \theta_n \} \\ (d\phi/dl)_1 &= g\rho \{ \sin \theta_1 + (dz/dl)_1 \cos \theta_1 \} \end{aligned} \quad (11)$$

By combining (7), (8), and (11), and remembering that even for slopes as steep as 1 in 3 the value of  $\cos \theta$  differs but little from unity, we get finally

$$(dz/dl)_n = n(dz/dl)_1 \quad (12)$$

for

$$z_n = z_1$$

This is satisfied by the solution

$$\begin{aligned} z_1 &= f(l) \\ z_n &= f(nl) \end{aligned} \quad (18)$$

where  $f(l)$  expresses the shape of the upper boundary for the slope  $\theta_1$  and is a function which need not be further specified for our present purpose. Equation (18) expresses the result that we have demonstrated experimentally, but since the argument involves approximations and is invalid in the vicinity of the drain, the solution itself can be regarded as only approximate.

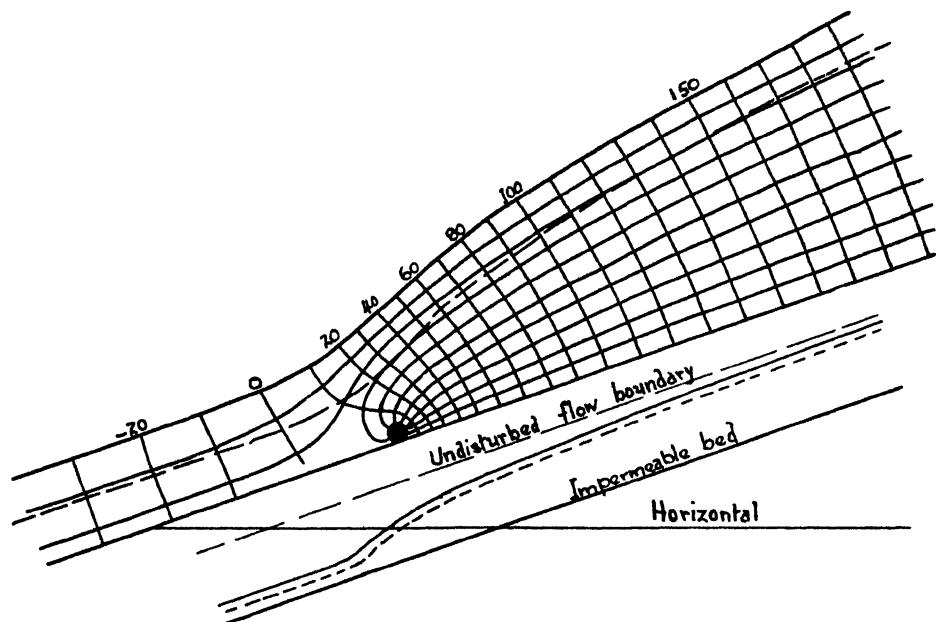


FIG. 8. SAME AS FIGURE 5, BUT WITH THE DRAIN LAID ON THE BED

The zero equipotential is not completely traced, since it is impossible to locate it with accuracy in the extensive area at effectively zero potential, just below the drain.

Although theory indicates that a surface of seepage must, in principle, develop on the uphill bank of an open ditch, our curves show that it cannot be detected for bed gradients up to 1 in 30. This is qualitatively in keeping with the findings of workers who have studied dam problems. If a ditch is dug down to the impermeable bed, the conditions on the uphill side somewhat resemble those in an earthen dam, the bed gradient being comparable in its effects with the ratio of height to thickness of the dam. Muskat (6) and Casagrande (1) have indicated how the surface of seepage on the downstream face of a dam shrinks as this ratio decreases. They were concerned with dams on horizontal beds, but their results may be interpreted for sloping beds by regarding an intermediate streamline as the bed and assuming, as an approximation, that no major changes will be brought about by flattening out this bed to a plane surface.

Only one drain diameter has been considered in this paper. The reason is that agricultural drains can only rarely be placed at depths exceeding 2 meters (more than 6 feet), and our curves, interpreted on this or a greater scale, correspond to a drain diameter of 4 inches or less. Nobody is likely to be interested in a drain diameter of less than 4 inches. At the same time, the control exerted by such a drain is complete at slopes up to 1 in 30 (so that no drain, however large, can do more) and not far short of complete at a slope of 1 in 3. There can be little occasion for draining slopes greater than 1 in 3 in ordinary agricultural experience. Hence there is as little point in repeating the work for larger drains as there is for smaller.

#### SUMMARY

Results are presented showing the water table and flow net in land protected against foreign water by pipe drains and open ditches. Two different gradients of impermeable bed have been considered, namely, 1 in 3 and 1 in 30, with drains either on the bed or at half the depth of the undisturbed flow zone. The surface of seepage, if any, and the capillary fringe have both been taken into account. At the less steep slope, control of foreign water is complete with a pipe drain 4 inches in diameter or with an equivalent ditch. At the steeper slope, both types of drain are equally effective, neither being completely so, when the floor of the drain lies midway between the bed and the boundary of the undisturbed flow. When the drain floor coincides with the bed, the ditch is obviously a complete interceptor, but the pipe drain permits an additional 10 per cent of the incident flow to pass to the downhill side. It is shown that, in accordance with an approximate theoretical treatment, control on the uphill side is exerted to a distance which is inversely proportional to the gradient of the bed. The surface of seepage on the uphill bank of an open ditch is shown to be negligible except at the steeper slope.

#### REFERENCES

- (1) CASAGRANDE, A. 1937 Seepage through dams. *Jour. New England Waterworks Assoc.* 51: 131-172.
- (2) CHILDS, E. C. 1943 The water table, equipotentials, and streamlines in drained land. *Soil Sci.* 56: 317-330.
- (3) CHILDS, E. C. 1945 The water table, equipotentials, and streamlines in drained land: II. *Soil Sci.* 59: 313-327.
- (4) CHILDS, E. C. 1945 The water table, equipotentials, and streamlines in drained land: III. *Soil Sci.* 59: 405-415.
- (5) HOPF, L., AND TREFFFTZ, E. 1921 Grundwasserströmung in einem abfallenden Gelände mit Abfanggraben. *Ztschr. Angew. Math. u. Mech.* 1: 290-298.
- (6) MUSKAT, M. 1935 The seepage of water through dams with vertical faces. *Physics* 6: 402-415.

## BOOKS

*Colloid Chemistry.* Volume 6. Collected and edited by JEROME ALEXANDER. Reinhold Publishing Corporation, New York, 1946. Pp. 1215. Price \$20.

This volume contains 71 papers by selected international contributors. These articles have to do mainly with the applications of colloidal chemistry in industry. Of the total, one article is on nuclear fission and atomic energy, and 32 deal with synthetic resins and plastics. Two articles of special interest to workers in soil science are "Colloid chemistry of clay minerals and clay films," by E. A. Hauser and D. S. le Beau, and "Principles and practice of soil stabilization," by Hans F. Winterkorn. Another article of special agricultural interest is "The colloid chemistry of insecticides," by O. A. Nelson and C. M. Smith.

Many of the other papers are of considerable interest to those who are concerned with applications of colloid chemistry to soils and plants, since they offer suggestions that might readily be applied to these fields. Some 4000 references are contained in the volume. Every scientific library should add this volume to the five that have preceded it, and many physical chemists will want it closer at hand.

*¿Cómo se Nutren las Plantas?* By CAMILO CIRANNA. Jorge Best, Mendoza, Argentina, 1946. Pp. 116.

This book is concerned with the various concepts on "plant feeding" and the fundamental principles upon which they are based. To these the author adds some further electro-osmotic concepts and comments on their application in connection with fertilizer practice. The conclusions are presented in Spanish, English, German, French, and Italian.

*Croissance des Végétaux Cultivés.* Third Edition. By ALBERT DEMOLON. Dunod, Paris, 1946. Pg. 362, figs. 96.

This is a companion volume to *La Dynamique du Sol*, third edition, published in 1944 (reviewed in SOIL SCIENCE 61: 191). The book is divided into four parts: the physical, chemical, and biological factors affecting plants and the growth and yield of crops. The several chapters deal with climate, atmosphere, roots, water, mineral nutrition, nitrogen, phosphorus and sulfur, potassium, calcium and magnesium, toxicity, soil biology, laws of growth, NPK fertilizers, crop quality, field plot techniques, and methods of analyses of plants. The new features in this edition are the greater emphasis on factors affecting growth and on ecological relationships. In addition, several new observations arising out of a scarcity of fertilizer materials during the war are presented.

*L'Evolution Scientifique et l'Agriculture Française.* By ALBERT DEMOLON. Flammarion, Paris, 1946. Pp. 325.

The purpose of this book is to trace the history of scientific agriculture in France from the days of J. B. Boussingault to the present. The several chapters deal with the evolution of French agriculture, the agricultural institutions of

France, agronomic research, climatological effects, the pedological approach, soil colloids, techniques of fertilization, factors affecting soil fertility, cultural implements, plant breeding, disease control, colonial agriculture, agronomy in relation to animal husbandry, and some considerations on agricultural policy. This is a popular rather than a technical presentation of the problems involved.

*Field Manual of Soil Engineering.* Revised. By CHARLES M. ZIEGLER. Michigan State Highway Department, Lansing, 1946. Pp. 304. Price, \$2.

The purpose of this manual is to describe the techniques developed in adapting the science of pedology to highway engineering. It presents a brief glacial history of Michigan, a classification of the soils of that state, the elements of highway design and construction, and the tests that are applied to determine the physical properties of soils as related to the needs of highway engineers. On reading it, one is impressed that the findings of the pedologist are contributing to the advancement of highway science.

*Food and Irrigation Problems.* By RAO BAHADUR N. S. JOSHI. Aryabhushan Press, Poona, 1945. Pp. 38. For private circulation only.

The problems presented in this volume are those affecting India in general and Bombay in particular. They deal with 15,700,000 square miles of land and a population of 388 million people, a large proportion of whom do "not get enough food to eat." The author points out that although India is an agricultural country, it has to import 1.5 million tons of rice annually to supplement its normal food production of 51.5 million tons. The population is expected to increase at the rate of about 1 per cent a year. Apparently the Malthusian principle is operating in India, and the author has attempted to outline a program of land reclamation and improvement that will meet the minimum needs of the people. His primary point has to do with increasing by 125 million acres the area under irrigation from canals, tanks, wells, and other means. Appendixes A to S, inclusive, contain some very interesting data on population, food supplies, food shortages, and irrigation costs.

*Glycerin.* By GEORGIA LEFFINGWELL AND MILTON A. LESSER. Chemical Publishing Company, Inc., Brooklyn, New York, 1945. Pp. 259. Price \$5.

Glycerin has so many important uses that 15 scientific and industrial organizations united, in 1936, in a formal cable to the King of Sweden on the highly important contribution of the chemist, Scheele, in discovering the substance in 1779. This book deals with the commercial applications of glycerin in the adhesive, cleaner, electrical, explosive, leather, lubricant, metal, packaging, paper, plastic, printing, paint, rubber, textile, tobacco, glass, cosmetic, beverage, food, and pharmaceutical, industries and in the fields of photography, agriculture, medicine, dentistry, veterinary science, and optometry. The readers of *SOIL SCIENCE* will be particularly interested in the chapter on agriculture, in which glycerin is credited with having value as a stimulant for germinating seeds and for transplants, for coating tree wounds, and as a solvent for insecticides and fungicides.

*Nitrogen in the Life of Plants and in the Agriculture of the U.S.S.R.* By D. N. PRIANISHNIKOV. The Academy of Science of the U.S.S.R., Leningrad, 1945. Pp. 197, figs. 35.

This book deals with the sources of nitrogen of plants and the conditions under which the ammonium and nitrate forms are most effective. It is, in effect, a summary of the author's well-known findings in this field of study, together with some suggestions as to their application in practical agriculture. The text is in Russian, except for the appended outline of contents.

*Phosphates and Superphosphates.* Second Edition. By A. N. GRAY. Interscience Publishers, Inc., New York, 1943. Pp. 416, illus. 37. Price \$7.

This book presents a record of the dates of beginning development of the world's phosphate deposits, a discussion of the methods by which phosphates are prepared for market, and a statistical summary of production, imports, and exports of both the phosphate rock and the products derived from it. The statistical records are complete up to and including 1939. The book contains a great deal of material that is useful to those concerned either with the manufacture or the use of phosphates. The historical and statistical features are of special value for reference purposes. Certain of the more recent technical developments, such as the ammoniation of superphosphate, are not mentioned.

*Research and Regional Welfare.* Edited by ROBERT E. COKER, with a foreword by Louis R. Wilson. The University of North Carolina Press, Chapel Hill, 1946. Pp. 229. Price \$3.

The papers reproduced in this volume were presented during a Conference on Research and Regional Welfare as part of the Sesquicentennial Celebration of the University of North Carolina at Chapel Hill. The subjects cover some of the more important of the researches of the past, of those that are now under way, and of those that should be undertaken. Among the speakers were R. Gregg Cherry, Frank Porter Graham, Wilson Compton, Wilbur A. Lazier, Raymond R. Paty, James Stevens Simmons, D. C. Allen, Avery Craven, Milton H. Fies, Reuben B. Robertson, David E. Lilienthal, and Georges F. Doriot. From the point of view of agriculture, the paper on research in nutrition, by Russell Wilder of the Mayo Clinic, and that on the Southern Farmer, by George J. Wilds of Coker's Pedigreed Seed Company, are of most interest. The excellent paper on fisheries by Harden F. Taylor, former president of the Atlantic Coast Fisheries Company, merits special mention. The university is to be congratulated on having so well reported on the opportunities for research for the betterment of the social and economic life of the nation, with special reference to the South.

*Statistical Methods.* Fourth Edition. By GEORGE W. SNEDECOR. The Iowa State College Press, Ames, 1946. Pp. 485. Price \$4.50.

The purpose of this book is that of so simplifying the statistical method as to make it a usable tool in the hands of the man who has little knowledge of or little time for the more elaborate mathematical considerations on which the method is

based. The scope has been enlarged to include greater emphasis on conditions for validity, experimental design, and samplings, treatment of correlation, and methods for proportionate subclass numbers. Of the 17 chapters, 9 have been selected as a short course. An attractive feature of the book is the system of numbering topics, tables, examples, and figures. Thus "Table 8.2" means the second table in chapter 8. A tremendous amount of constructive effort has gone into rewriting this book. The contents are presented in very readable form, with sufficient detail to make the subject comprehensive to anyone who is prepared to invest a reasonable amount of time in its study.

*Surface Active Agents.* By C. B. F. YOUNG AND K. W. COONS. Chemical Publishing Co., Inc., New York, 1945. Pp. 381, figs. 41. Price \$6.

This book presents the theory of surface tension and offers suggestions for its practical application in various industries. Methods and apparatus for the determination of surface tension are described. A long list of wetting and other surface agents is presented, with their structures and uses and the names and addresses of their manufacturers. The applications have to do with emulsions, plating, cosmetics, leather, flotation, inks, textiles, cutting oils, adhesives, foods, lubrication, and soldering. A bibliography is appended at the end of each of the 15 chapters. Those concerned with this field of study will find this book a very handy and useful reference.

*X-Rays in Practice.* By WAYNE T. SPROULL. McGraw-Hill Book Company, Inc., New York, 1946. Pp. 615. Price, \$6.

This book may be said to commemorate the hundredth anniversary of the birth of W. C. Röntgen, who discovered x-rays on November 8, 1895. It presents a history of x-rays, the fundamental principles involved, the equipment employed in their use, and the applications that are being made of x-ray analysis in medicine, industry, and pioneering research. The author acknowledges the contributions of many colleagues in assembling the material contained in the book. The final chapter deals with the subject of electron diffraction and its applications. The index is especially well prepared and permits of finding ready answers to most of the questions one might have on any phase of this subject. The book will fill a widespread need for exact information on this highly important subject.

THE EDITORS.





S. N. WINOGRADSKY, AT HIS LABORATORY DESK, AT BRIE, IN 1923

# SERGEI NIKOLAEVITCH WINOGRADSKY<sup>1</sup>

September 1, 1856–August 31, 1946

## THE STORY OF A GREAT BACTERIOLOGIST

SELMAN A. WAKSMAN

New Jersey Agricultural Experiment Station, Rutgers University

*On pourra parler de microbiologie agricole comme d'une science constituée, dont l'importance pour l'agriculture ne saura être estimée assez haut.—S. N. Winogradsky.*

### BIOGRAPHICAL

Sergei Nikolaevitch Winogradsky was born September 1, 1856, in the city of Kiev, in the center of the Ukraine region, Russia. His father, Nikolai Konstantinovitch Winogradsky, was a native of Bessarabia, the most southwestern province in Russia. His mother, Natalia Viktorovna Skoropadskaya, came from a famous old Ukrainian Getman family in the State of Tchernigov. His father was graduated from the faculty of law of the University of Kiev and entered government service. Tiring of this, he engaged in banking and in due time became Director of the Private Commercial Bank of Kiev, the first of its kind in that region, and later also of the newly opened Agricultural Bank, the first in the whole Ukraine. The construction of the Southeastern Russian railway and the banking connections brought him considerable income. As a result, he purchased several estates in the Podol region, in the neighborhood of the town of Gorodok, of Grudok, state of Kamenetz-Podolsk, not far from the city of Proskurov. While Sergei was still a child, an old, disused beet-sugar factory connected with one of the estates was sold to a corporation, a pioneer type of organization in that region, formed to modernize and operate the plant, the majority of the shares being retained in the family. The various estates were then combined into one, and the soil brought under intensive cultivation, special attention being paid to seed selection.

Winogradsky himself was born in a large old family house, with an extensive garden, overlooking the river Dnieper. He had two brothers and one sister. His older brother, Alexander Nikolaievitch, received a law education at the University of Kiev. Subsequently, he became intensely interested in music, and while occupying an official position in St. Petersburg, found time to study the theory of music at the Conservatory. Later, while filling his father's position as Director of the Agricultural Bank in Kiev, he began, as an amateur, to conduct an orchestra. After a certain amount of specialization, he became one of the leading artist-conductors in that region and subsequently appeared

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EDITOR'S NOTE: This tribute is published to commemorate the ninetieth anniversary of the birth of Dr. Winogradsky, who for many years has been a consulting editor of *Soil Science*.

on the stages of Paris, Berlin, and Vienna, not to earn money, but to introduce Russian music, at that time still little known in Western Europe.

Winogradsky's childhood was spent in the city of Kiev proper. He entered the second class of the Second Classical Gymnasium at the age of ten and graduated from the Eighth Class, at the age of sixteen, with a gold medal. In order to prove his hearty dislike of the bureaucratic atmosphere of the gymnasium, which was characteristic of the Russian middle schools in general, he sold his medal immediately.

In 1873, Winogradsky entered the University of Kiev, where he spent two years, at first studying law like his father and his elder brother. Tiring of legalistic studies, he soon transferred to the division of natural sciences, in the physico-mathematical faculty. This period at the university left no deep impression upon his development. Although he visited the lectures regularly and passed all his examinations on time, the whole atmosphere of this institution of higher learning offered but little attraction for him. It should be recalled that this period marks the beginning in Russia of extensive revolutionary activities, in which the students of the universities became actively engaged. Winogradsky, being of a scientific turn of mind and coming from a wealthy family, took little interest in student politics. Several of his early friends, however later became prominent revolutionists.

The number of students in the natural science division of the university was very small, usually three to five. The practical exercises were poorly organized, with little analytical chemistry and still less of other sciences. This background could hardly prove stimulating to an inquiring mind. As is frequently the case, it had an opposite effect; it resulted in producing a state of bodily weariness and of complete spiritual disappointment. Winogradsky became so discouraged in his university work that, upon entering the third year, he ceased altogether to visit the lectures and laboratory exercises and lost all interest in further studies. He finally decided, in spite of protests from the family, to drop out of the university curriculum altogether and find an outlet for his energy elsewhere.

Science, especially as it was organized at the University of Kiev, having thus failed to satisfy his natural curiosity, he became interested in music. This led him to the Conservatory of Music at St. Petersburg, where he was admitted to the piano classes of the most brilliant music teacher of that time, Professor Leshetitski, who later became the teacher of Paderewski and of many other famous musicians. Winogradsky developed a deep interest in his work at the conservatory, which left an indelible imprint upon his personality. He soon became convinced, however, that "aesthetic emotions alone, without any activity of the brain, could not satisfy him for very long." Furthermore, the early departure of Leshetitski for Vienna hastened the decision that the conservatory did not offer him the proper field of activity. Regretting his mistake in changing from the field of science to that of art, he decided to return to university work and make another attempt to obtain a scientific training, not through mere formal attendance of lectures and laboratory exercises, but through the pursuit of deeper interests that could satisfy the strivings of his mind.

In 1877, Winogradsky entered the natural science faculty of the University of

St. Petersburg. Since he was particularly anxious to receive a thorough preparation in chemistry, he began immediately to study analytical chemistry, under the leadership of Professor Menshutkin. The faculty of this university consisted at that time of a group of some of the most brilliant and enthusiastic scientific minds ever gathered at any one time at a Russian institution. Winogradsky had thus an opportunity of attending lectures by such outstanding representatives of their respective fields as Mendeleiev, Menshutkin, Butlerov, Inostrantzev, Beketov, A. S. Famintzin, Kessler, and Wagner. Beginning with the third year of university work, it was necessary to select a special field of study and specialize in that field. Winogradsky decided upon the subject of plant physiology, for which he had a particular liking. For this work, he entered the laboratory of the famous Russian botanist Famintzin.

On his graduation from the university in 1881, with a diploma and first candidature in science, he was invited by the faculty, on the recommendation of Famintzin, to remain as a candidate at the University, in preparation for a professorship. He accepted and, attracted by the epoch-making discoveries of Pasteur and the mycological investigations of deBary and Woronin, began immediately to study microorganisms and their activities.

In 1879, while still an undergraduate, Winogradsky married Zinaida Alexandorovna Tichotzkaia, with whom he led a happy married life, lasting for exactly six decades. They had four daughters, three of whom are still living.

Beginning with his graduate study at the university, the life and scientific activities of Winogradsky can be divided logically into seven periods, as outlined, in general, by his famous assistant Omeliansky:

1. *The first St. Petersburg period (1881-1884)*, when his interest in science matured. Though this may be considered as still a period of intensive training, Winogradsky began and completed his first scientific problem, which proved to be highly successful. During this period, he worked in the laboratory of plant physiology of the university.

2. *The Strassburg period (1885-1888)*. Here he carried out his first investigations on the autotrophic bacteria. The problems dealing with the sulfur and iron bacteria were begun and completed at the botanical laboratory of the university under deBary.

3. *The Zürich period (1888-1891)*. The study of the organisms concerned in the process of nitrification was begun and nearly completed at the agricultural faculty of the polytechnic and at the hygienic laboratory of the university.

4. *The second St. Petersburg period (1891-1905)*. This began with research activities and ended in administration work, the latter being largely responsible for his subsequent temporary retirement from both. The most important research problems dating to this period concerned the fixation of atmospheric nitrogen and the retting of flax. The work was done at the Institute of Experimental Medicine.

5. *The period of transition and rest (1905-1922)*. These 17 years were spent by Winogradsky on his estates in the Ukraine, away from scientific work. As a result of political upheaval following the World War and the revolution, he was eventually forced to leave his native country forever. After a few months spent in Jugoslavia, he finally arrived at the Pasteur Institute in France.

6. *The active Brie-Comte-Robert period (1922-1940)*, which signalized a return to scientific work. The problems considered were largely connected with the broad aspects of soil microbiology. This work was done in the Division of Agricultural Microbiology of the Pasteur Institute.

7. *The period of forced retirement (1940-1946)*.

*The first St. Petersburg period (1881-1884)*

The first four years of the scientific life of Winogradsky were still largely preparatory in nature, in regard both to scientific procedure and methods and to an understanding of the literature in the selected subject. The newborn science of microbiology attracted Winogradsky from the very beginning. Although the work of the botanists deBary, Woronin, and Brefeld, in the field of mycology and plant pathology, laid the foundation for our knowledge of fungi, although the investigations of Ferdinand Cohn and his pupil Robert Koch were the basis for bacterial classification and for an understanding of bacterial species, it was Pasteur's brilliant contributions to microbiology that principally attracted Winogradsky's attention. Winogradsky familiarized himself with the ideas of the various investigators in the field of microbiology, but he took the trouble to repeat in detail only the experiments of Pasteur. The latter's classical studies on fermentations directed him to a new field of investigation and left a profound impression upon his future work.

Winogradsky's first independent effort in the field of microbiology supplemented the investigations of various fermentations with morphological and cultural studies of the organisms concerned in these processes. He made a careful study of the influence of environment and conditions of nutrition upon the changes in form and characteristic growth of the wine-yeast, *Mycoderma vini* Desm. By cultivating this organism from a single cell (Hansen's method), using small culture vessels (Geissler's chamber) connected with a microscope camera, he was able to observe the growth of the organism under changing conditions of nutrition, whereby not only was the culture medium modified but the gas tension of the culture was also controlled. He was thus able to make a study of the influence of organic and inorganic nutrients as well as of oxygen tension upon the growth of the specific organism. He found that magnesium is essential for growth, whereas calcium can be omitted from the medium without any injurious effect. Nägeli's conclusions that rubidium can take the place of potassium in the culture were confirmed; the latter's assertion however, that this is also true of calcium was not substantiated.

These experiments may well be considered as among the first careful investigations ever made on the influence of environment upon the growth of lower forms of life in pure culture, under controlled experimental conditions, and also on the changes and variations of individual cells of microorganisms, followed continuously by direct microscopic observation. The results of these investigations were summarized and presented in a paper before the Botanical Section of the University, on December 25, 1883. The botanist Borodin, who was present at the meeting, summarized this paper in the *Botanisches Centralblatt*, and added the following footnote: "The writer admired the purity of the cultures exhibited by the speaker in Famintzin's laboratory." The extensive series of results obtained in these investigations were never published. Only a brief preliminary abstract appeared in the *Contributions of the St. Petersburg Society of Natural Science for 1884*.

In the fall of the same year, Winogradsky obtained the degree of master of science in botany from the university. Soon, however, the illness of his wife forced him to leave the cold northern climate and settle for a time in the south, in Crimea. He established a small laboratory in his home where he continued the investigations of the morphology of the microbial cell, using the method of Strassburger.

The conditions for continuing his scientific investigations in Russian universities did not prove sufficiently favorable to satisfy Winogradsky. As a result, he decided to go abroad, in order to continue his work under the more favorable conditions which only the laboratories of Western Europe could offer. For this purpose, he selected the botanical laboratory of the University of Strassburg. At the head of this laboratory was the famous cryptogamic botanist, Anton de Bary, whose ideas on the morphology and physiology of microorganisms well suited the tendencies already shown by Winogradsky. In November, 1885, Winogradsky left for Western Europe.

#### *The Strassburg Period (1885-1888)*

During the ninth decade of the last century, microbiology was passing through a period of controversy and heated discussion between two schools of thought. On the one hand, F. Cohn and his associates, strong adherents of the monomorphistic theory, insisted upon the stability or constancy of microbial species; on the other hand, Nägeli, Zopf, and their schools, usually referred to as the "pleomorphists," insisted upon the variability of microorganisms.

Winogradsky's first problem at the botanical laboratory in Strassburg was to investigate Zopf's ideas concerning the pleomorphism of "higher bacteria." Several species of such bacteria, namely, *Beggiatoa alba*, *Begg. roseo-persicina*, *Cladotrichia dichotoma*, and *Crenothrix polyspora*, were used by Zopf to confirm his pleomorphistic conceptions, which were based largely upon interpolations made between intermittent observations. De Bary did not have much confidence in these results; he was very happy, therefore, to see that Winogradsky was interested in the problem and ready to study it. The results obtained led directly to the refutation of Zopf's ideas. *Cladotrichia dichotoma* was found to pass through a regular life cycle, instead of being subjected to a chaotic state of change, as affirmed by Zopf. The various other cultures of bacteria used by the latter in his investigations proved to be mixtures of organisms and not pure cultures of bacteria.

In carrying out the investigations on the morphology of thread-forming bacteria, which proved to be very exhausting at times, Winogradsky became particularly interested in their specific metabolism. He found that *Beggiatoa* represented interesting material for physiological studies. This was especially true of the function of hydrogen sulfide in the growth and energy utilization of this organism; the role of the sulfur found within its cells also represented a profound enigma. De Bary could never understand, for example, why this organism required so much sulfur for its nutrition. Two years passed in this study, but the problem still remained unsolved. Winogradsky became impa-

tient; he could not find any analogy whatsoever between the physiological behavior of this organism and that of other bacteria. One day, while he was out walking on the street, the entire solution of the peculiar nutritional characteristics of *Beggiatoa* and other similar bacteria presented itself. All the observations previously made suddenly assumed new significance and dovetailed into a complete system in the form of a new phase of bacterial physiology, distinct from that of the majority of bacteria. Once the major aspect of the problem was understood, the investigations were soon completed, and the results appeared in print in 1887.

The process of formation of hydrogen sulfide in nature, and its role in the cycle of sulfur in general were at that time very little understood. The common belief prevailed that hydrogen sulfide is produced through the action of certain so-called sulfur bacteria upon sulfates; these organisms were regarded as the agents responsible for the formation of hydrogen sulfide and its appearance in sulfur springs. Winogradsky demonstrated that these bacteria bring about the disappearance rather than the formation of hydrogen sulfide, by the oxidation of the latter to sulfuric acid. These investigations resulted in a complete elucidation of the function of sulfur bacteria in nature and of the specific physiology of these organisms. It is true that Engler and particularly Hoppe-Seyler spoke of the energetics of the process of sulfate reduction and of hydrogen sulfide oxidation; the latter further observed that *Beggiatoa* will die when air is excluded. It was Winogradsky, however, who, by his unique and clearly defined methods, definitely established the specific physiology of these organisms, namely, the role of sulfur as a source of energy for *Beggiatoa* and related forms, which enables them to utilize the carbon of carbon dioxide and bicarbonates. He states emphatically in his paper:

Auch ist die Oxydation des Schwefels die einzige Energiequelle . . . Es muss für die S-Bakterien ohne Bedeutung sein, welcher Art die ihnen zur Verfügung stehenden C Verbindungen sind, wenn sie nur C in assimilierbarer Form enthalten. Diese Körper brauchen nicht "gute" Nährstoffe zu sein, d.h. Verbindungen, bei deren Zerfall oder Verbrennung viel Wärme frei wird.

In the history of bacteriology, Winogradsky's name will be forever connected with the whole group of autotrophic bacteria, which he later designated as "anorgoxydants." His first outstanding contributions in bacteriology dealt with the three most important representative groups of these bacteria, the sulfur-oxidizing organisms, the iron bacteria, and the nitrifying bacteria. It is of interest to note that in the declining years of his life, nearly half a century after these first investigations were carried out, Winogradsky returned to the study of the same groups of organisms, as if to fill in the gaps which were left through the long period of his retirement and interest in other problems.

In 1888, Winogradsky completed his work on the morphology of the sulfur bacteria, and the results were published in the form of a separate monograph printed in Leipzig. The same year, he also completed his study of the iron bacteria, another group of organisms which served as a basis for Zopf's pleomor-

phic assumptions. In attempting to check the latter's results on the morphology of *Crenothrix polyspora*, Winogradsky became interested in the physiology of iron bacteria in general. The same nutritional concept that was applied to the sulfur bacteria was further developed in the case of the iron bacteria. In this work as well, he showed that the specific physiology of these bacteria places them far apart from the great majority of other organisms in nature, the heterotrophic forms. In these investigations he first utilized the elective culture method for the study of microorganisms. This method, introduced through the work of Beijerinck and Winogradsky, proved to be one of the most valuable tools in the isolation of various groups of bacteria, especially those that are highly specific in their nutrition.

It seemed that, after Winogradsky had so clearly blazed the way for the study of the autotrophic bacteria in general and that of iron bacteria in particular, later workers would have no difficulty in following the trail and carrying on. Unfortunately this was not the case. Many of the subsequent workers on this group of bacteria, notably Molisch, Ellis, and Naumann, entirely confusing the original ideas of Winogradsky, included under iron bacteria all organisms capable of bringing about, directly or indirectly, the precipitation of iron, without reference to the role of the process in their physiology. Fortunately, Winogradsky himself lived long enough to clear up, thirty-four years later, the accumulated misunderstandings in connection with the study of this group of organisms.

The publication of the results of the investigations on the sulfur and iron bacteria attracted, in the scientific world, considerable attention to Winogradsky as a talented investigator who not only possessed original ideas but was also able to utilize them for widening knowledge of certain very obscure forms of life.

Toward the end of 1887, de Bary suddenly became ill and soon died. With de Bary's death, Strassburg lost for Winogradsky its chief attraction as a scientific center. When he completed the studies in progress, he decided to leave that city. He returned to Russia in 1888, visiting Kiev and St. Petersburg. Omeliansky has suggested that Winogradsky intended to become connected with one of the Russian universities, but he took no action to further any such plans, if these existed at all. He had left his family in Germany, where he soon rejoined them. He decided to settle in Zürich, which at that time was an outstanding scientific center.

#### *The Zürich Period (1888-1891)*

During the years spent at the University of Strassburg, Winogradsky had not neglected his training in various branches of chemistry. This he continued during most of the first year in Zürich, with particular emphasis upon agricultural and organic chemistry, especially methods of determining various forms of nitrogen, under the leadership of E. Schulze and Hantsch. Winogradsky was thus preparing himself for work in microbiology from a broad biochemical point of view and for the study of the function of microorganisms in the cycle of

elements in nature. Unlike most of the bacteriologists of that period, he was not satisfied to devote his energy to the enumeration of bacteria and to isolation of organisms responsible for certain processes.

While he considered work on a new problem in Zürich, his attention was still occupied with certain matters pertaining to his previous investigations. These were a direct result of polemics with other bacteriologists. He wrote two discussion papers at that time, one dealing with the sulfur bacteria, in reply to Olivier, and the other with the question of bacterial pleomorphism, in reply to Metchnikov.

In 1889, Winogradsky was ready to begin a new series of investigations resulting in the solution of a highly important and most difficult problem, the process of nitrification, which was to become forever connected with his name. For this purpose, he established himself at the hygienic laboratory of the university. The ninth decade of the last century will be memorable in the history of nonmedical bacteriology as a period connected with the search, by both agricultural chemists and bacteriologists, for the agents responsible for this process of nitrification. Schlösing and Müntz definitely established in 1877 that this process is biological. All attempts, however, by a number of investigators to determine the nature of the organisms concerned in the process and to obtain these organisms in pure culture failed completely. This failure was due primarily to the fact that the specific physiology of the organisms was little understood. The nearest approach to the solution of this problem was made by Frankland, Munroe, and especially Warington, in England. Although these investigators carried out a large number of experiments which tended, unfortunately, to confuse the microbiological aspect of the process, they established the fact that nitrification proceeds in two stages, the ammonia being oxidized to nitrite and the nitrite to nitrate, and that organic nitrogenous compounds are broken down to ammonia before nitrate formation can take place. Other bacteriologists went so far as to say that since all bacteria presumably develop on the gelatin plate and since these hypothetical nitrifying organisms could not develop at all upon this plate, *ipso facto*, they do not exist. Heraeus claimed that all bacteria, including such forms as *Cholera vibrio* and *Micrococcus prodigiosus*, can produce nitrates; he concluded, therefore, that no special "ferment nitrique," as understood by the French workers, exists. It is true, of course, that this "ferment nitrique" ascribed to Schlöesing and Müntz and featured for many years in all textbooks, was a pure myth, since it was never studied by these investigators.

Winogradsky was free from those prejudices concerning bacterial nutrition that dominated many of the bacteriologists trained primarily in medical schools. Furthermore, he had the distinct advantage of having already worked with and thoroughly understood the metabolism of two other groups of bacteria which required inorganic substances as sources of energy. It soon became apparent that here was another group of organisms possessing a similar physiology. After a preliminary experiment, in which he added organic substances to his culture media, he conceived the idea that it might be best to exclude all traces

of organic matter. Although such exclusion had been practiced previously by Heraeus, who noted that, in the absence of glucose, the process of nitrification proceeded at a more rapid rate than in its presence, these observations brought no tangible results in advancing our knowledge of the organisms concerned. Winogradsky, in his characteristic role of careful observer and expert in devising new and suitable methods, succeeded where others failed.

A series of investigations, concluded with amazing rapidity (within the year 1889-1890), yielded perfect results. All that subsequent investigators of the subject of nitrification could do was to confirm these results. The mechanism of the process was so completely and carefully studied during this and the subsequent year, that very little that was new was added during the next forty years. Thus was solved one of the most difficult problems of general microbiology, dealing with a process highly significant in plant nutrition and in soil transformations, in sewage disposal, and in marine life. It placed the name of Winogradsky in the front rank of scientific workers, affording him a prestige which has since remained unchallenged.

The observations of Warington and others, that the process of nitrification is brought about in two stages, were confirmed. Winogradsky went much further and succeeded in establishing the fact that two distinctly different groups of organisms are concerned in these processes. The specific bacteria were isolated in pure culture and their physiological characteristics studied. One group was shown to oxidize ammonium salts to nitrite; several representatives of this group were isolated and placed in the newly created genera *Nitrosomonas* and *Nitrosococcus*. The second group was shown to oxidize nitrite to nitrate; only one representative was described in 1891, under the genus *Nitrobacter*. As a result of the oxidation of the ammonium ion to nitrite and of the nitrite to nitrate, energy is liberated. This energy is utilized by the specific bacteria for the assimilation of carbon dioxide from the atmosphere and for the building of cell substance. The process of carbon dioxide assimilation by the nonchlorophyl-bearing bacteria, which has since become known as *chemosynthesis*, is comparable to that of photosynthesis in the chlorophyl-bearing algae and higher plants. These two groups of bacteria are obligate autotrophic, that is, they are totally unable to use any other source of energy except that liberated in the specific oxidation processes. Organic matter is unnecessary for their metabolism; actually the presence of soluble organic substances was found to be injurious to their development, especially to that of the nitrite-forming organisms.

These investigations of Winogradsky were published in a series of papers in the *Annales de l'Institut Pasteur* in 1890-1891. A supplementary memoir, devoted largely to a study of the morphology of the organisms and certain supplementary physiological studies, such as the influence of organic substances, appeared in 1892, in the Russian *Archives de Sciences Biologiques*, in both French and Russian. A complete summary of Winogradsky's work on the nitrifying bacteria was prepared several years later, in 1902, for the third volume of Lafar's *Handbuch der technischen Mykologie*.

This work received universal recognition and aroused so much attention that several tempting offers of positions were immediately made to Winogradsky. The president of the polytechnicum in Zürich invited him to deliver a course of lectures at that institution, in the capacity of privat docent. In 1891, I. I. Metchnikov, another brilliant Russian bacteriologist, visited Winogradsky, at the request of Pasteur, and invited him to come to Paris to organize a bacteriological laboratory at the newly established Pasteur Institute. Winogradsky was on the point of accepting this attractive offer when another invitation reached him. An institute was then being organized in Russia to correspond to the Pasteur Institute. It was to be known as the Institute of Experimental Medicine in St. Petersburg. The organizer, Prince Oldenburg, offered to Winogradsky the position of chief of the division of general microbiology. It was very difficult for Winogradsky to decide between the offer from France and that from his native land. Had he accepted the first, he, like Metchnikov, probably would never have returned to his homeland, especially as he had little liking for Russian bureaucracy and Russian officialdom. However, his love for his country and the fear of becoming in time entirely expatriated prevailed, and he accepted the St. Petersburg offer. It must be remembered, also, that he had in Russia close friends and relatives with whom he shared a community of interests. The division of his father's estates about that time also influenced his decision, for Winogradsky himself received the estate near Gorudok which he had loved since childhood.

In March, 1891, Winogradsky left Zürich for Paris, to meet Pasteur and present to him the reasons for his final decision. He was well received by the great master in the field of microbiology, who repeated personally the invitation previously issued through Metchnikov. Winogradsky was highly impressed by the spirit of the institution in Paris and by the prevailing scientific atmosphere, but his mind could not be changed.

#### *The second St. Petersburg period (1891-1905)*

Arriving at St. Petersburg in the fall of 1891, Winogradsky was immediately appointed chief of the division of general microbiology of the Institute of Experimental Medicine. He was also made the first editor of the newly established journal *Archives de Sciences Biologiques*, published by that institute. Largely through his efforts, this journal has since become famous as the leading biological publication in Russia. Upon his return to Russia, the university of Kharkov conferred upon him the degree of doctor of botany *honoris causa*. Two years later he was elected to corresponding membership in the Russian Academy of Sciences.

The physiologist Ivan Petrovitch Pavlov, who soon received the Nobel prize, and the chemist, Nencki, who came to Russia from Bern, Switzerland, were active members of the institute from its organization. During the first few years their activities developed on a much wider scale than those of Winogradsky. Their laboratories became centers for medical students, who there prepared doctoral dissertations. Winogradsky's laboratory did not expand very materially, however, because of the more limited appeal of the subject of general

microbiology and the selective personality of Winogradsky himself. His work was further interrupted by the editorship of the Archives, "which took much time and was not without unpleasantness."

On settling in St. Petersburg, Winogradsky turned his immediate attention to another little-understood phase of microbiology, that of nonsymbiotic nitrogen fixation in nature. This process may be considered one of outstanding importance in the soil and in the continuation of life upon this planet. The general idea prevailed that the fixation of nitrogen is carried out in nature by two distinctly different agents or groups of agents, one living symbiotically with higher plants, and the other living in a free state outside the plant. The importance of those organisms responsible for the symbiotic nitrogen-fixation process, the nodule-forming bacteria, had already become established, largely as a result of the investigations of Hellriegel and Wilfarth in 1886. The agents effecting the fixation of nitrogen nonsymbiotically still remained unknown. It is true that Berthelot, having found in the early eighties that unsterilized soil samples gained in nitrogen whereas those that were sterilized did not, was led to claim that certain free-living soil bacteria are capable of fixing nitrogen. These claims were not based upon undisputed experimental evidence but were founded mainly upon logical speculations. The bacteria isolated for him by Guignard seemed to show certain tendencies to fix nitrogen. Very little importance, however, was attached to this work at the Pasteur Institute; Berthelot's efforts in that direction were looked upon as rather amateurish. Unfortunately, the great emphasis which he placed upon his conclusions and his scientific reputation as a great chemist were chiefly responsible for the general acceptance of his statements as facts.

Winogradsky took up this problem in 1892 and was successful in demonstrating, in 1893-1894, the existence in the soil of an anaerobic bacterium, *Clostridium pastorianum*, capable of fixing atmospheric nitrogen. Here, as well, the principle of elective culture was applied. Liquid media containing soluble carbohydrates as sources of energy and either free from nitrogen or containing only traces of this element in a combined form were employed. Each medium was buffered with calcium carbonate. An anaerobic, rod-shaped, spore-forming organism producing butyric acid and gas developed when the medium was inoculated with soil. This organism was isolated in pure culture, by the use of potato slants containing some calcium carbonate. It was found capable of fixing nitrogen in a medium free from combined nitrogen, usually to the extent of two or three milligrams per gram of sugar decomposed.

The *Clostridium* thus isolated was an obligate anaerobe. When the liquid culture was first inoculated with soil and kept under aerobic conditions, a pellicle developed on the surface, while the anaerobic organism grew below the surface of the liquid. The pellicle was produced by an aerobic bacterium, also capable of fixing nitrogen. Primarily interested in the anaerobic form, Winogradsky failed at that time to recognize the importance of this organism. It remained for Beijerinck to describe the aerobe eight years later under the name *Azotobacter chroococcum*.

These investigations, like the earlier ones of Winogradsky, attracted universal

attention. They were followed immediately by numerous studies throughout the world dealing with the same subject. It was thus generally believed that the problem of nonsymbiotic nitrogen fixation had been solved, just as had those of nitrification and of symbiotic nitrogen fixation.

Before many years had passed, numerous questions arose concerning the importance of these organisms in the cycle of life. The first problem was to determine the source of energy available to the bacteria in nature. Winogradsky himself tried to solve this problem, but he made little progress. It was not until nearly 40 years after the publication of the first paper that Winogradsky returned to these studies.

One of the most interesting characteristics of Winogradsky's scientific career, and one which prevented him from attacking a great variety of problems, was his paucity of students and his even greater paucity of assistants. His two major assistants during this period, V. A. Fribs and V. L. Omeliansky, were the only students whom he trained. Of these, Omeliansky was the only one to carry on Winogradsky's work for many years to come.

Fribs assisted Winogradsky in the study of the organisms concerned in the retting of flax. In this process, the pectin which holds the cellulose fibers together is destroyed by microorganisms. The common practice in Russia and in certain other flax- and hemp-producing countries is to stack the fibrous plants in heaps in the midst of slow-moving streams or rivers, cover them with soil or with river mud, and let them remain in that state for four to six weeks. This results in the loosening of the fibers; these are then washed and dried, after which they are ready for beating. The process is primitive and is accompanied by the pollution of the rivers and streams. Two groups of organisms are known to be concerned in this process, anaerobic and aerobic bacteria (the fungi as causative agents of flax retting in certain regions need not be considered here). The anaerobic bacterium was isolated by Winogradsky and Fribs in 1895 and was found to be capable of bringing about the process of retting in pure culture. Though this problem was not then carried to its practical end, recent extensive studies in Italy and elsewhere of the retting process carried out by pure cultures of bacteria seem to point to the greater importance of anaerobic organisms; these, as well as certain aerobic bacteria, are now utilized industrially for retting fibers under controlled factory conditions.

Omeliansky assisted Winogradsky in his studies on the physiology of the nitrifying and nitrogen-fixing bacteria. Later, in 1900, he took up, largely on his own account, the study of anaerobic cellulose-decomposing bacteria.

These investigations completed the early period of Winogradsky's scientific activities. It looked as if the end of his scientific career was approaching. It is of interest to analyze in detail the causes leading to this situation.

In 1898-1899, Winogradsky became ill with nephritis, following a severe attack of influenza. This served to interrupt his scientific work. Finding the severe northern climate of St. Petersburg very unhealthful, he began to spend much of his time on his estates in the Ukraine. Had that been the only interruption, he would have adjusted himself sooner or later in some way, but much

more disturbing conditions existed. The bureaucratic atmosphere of the Institute in which he carried out his work repelled him. He felt more like a western European and could not adjust himself to this stifling atmosphere, under the very shadow of the imperial court and of court life. Winogradsky was irked by constant friction with officialdom. Furthermore, the interests of the institute were largely medical. As a result, he did not feel at home in St. Petersburg. He did not enjoy life in the city and longed for his native Ukraine. It was a combination of these factors that finally led to his retirement into private life.

The question of giving up his position and the scientific work was not decided immediately, but only after several years. In the meantime, Winogradsky was to play an important role in the reorganization of the institute and in the growth of microbiology in Russia. In 1902, he was made director of the Institute of Experimental Medicine; he organized a scientific seminarium and continued as editor of the Archives. He was also a member of the Medical Council of the Empire and of the Scientific Committee of the Ministry of Agriculture. In 1903 he took an active part in the formation of the Russian Microbiological Society and served as its president during the first two years. He never accepted for personal use the salary allowed him by the institute, but laid it aside with the hope that out of the sums thus accumulating a new bacteriological laboratory might be built. These funds were later used for the construction of the Library of the Institute of Experimental Medicine.

In 1901, Winogradsky was elected an honorary member of the Moscow Society of Natural Science, and in 1902 a corresponding member of the French Academy of Sciences.

The break in his scientific career finally came in 1905, when he left the directorship of the institute, gave up his work as the head of the division of general microbiology, as well as all other official duties, and retired into private life, though his official separation from the institute did not take place until 1912. In addition to his poor health, which necessitated his going southward at frequent intervals, and his lack of adjustment to St. Petersburg officialdom, the Russo-Japanese war served as an added cause for his retirement. All the financial means of the country were mobilized for continuing this unfortunate war. Certainly no money was available for either science or education. All of Winogradsky's plans for a number of fundamental changes in the organization of the institute had failed. The financial difficulties which were a direct result of the war, and the general impossibility of accomplishing anything constructive at that period, served as the final cause of the break.

#### *The period of transition and rest (1905-1922)*

Upon retiring from active scientific work, Winogradsky settled permanently on his estates in southern Russia, in the State of Podol, where he devoted himself to the practical problems of farm management, as well as to his much-beloved music. His actual residence was divided between Russia, where he spent the

summers, and Switzerland, where he spent much of the winters in a villa, known as "Petit Cret," which he had built for himself near Clarens on Lac Leman.

On his Russian estates he developed farming to a high degree of perfection. An old forest of more than 1000 hectares was brought under a modern system of management. For this purpose, he made an extensive study of silviculture, in which he was assisted by Professor Dobrovolnikov and two students of the Forestry Institute. A large nursery was established, in which a number of valuable trees were grown. He took particular pride in this forest and to it became very much attached, as a result of his daily walks. He built stables for the raising of horses, organized a model dairy farm, and devoted much time to an orchard which he planted himself. He also took an active part in the management of the extensive beet-sugar factory, built by his father, as well as of several flour mills on his estates. His relations with the inhabitants of the town of Gorodok, adjoining his properties, were most cordial.

In this atmosphere, so well characterized by the great Russian writers Turgenev, Tolstoi, and Chekhov in their stories of the life of the Russian land-owners and of the Russian nobility, Winogradsky lived for almost a decade. Not of the Oblomov type, as depicted by Gontcharov, but of the active, westernized, progressive type, which was trying to help Russia and the Russian people to arise from the feudal system and take their place in the front rank of European nations, he seemed destined to spend the rest of his life in a state of plenty within a happy family circle. But fate had decided otherwise.

In 1914 the World War broke out. Several members of Winogradsky's family immediately entered the government war service in various capacities, the daughters as nurses and the sons-in-law as soldiers and engineers. Winogradsky himself raised on his extensive fields and in his fine stables foodstuffs and horses required by the armies, thus contributing his share to the war, in an economic way.

The rest is history, a large part of it, especially that pertaining to the role of the Ukraine, still remaining to be told. After three years of severest fighting, the war was lost, and down with the armies came the old system of Russian government. Mother Russia was shaken to her very foundations. The first revolution was followed by the second. This was accompanied by civil war which reached its fiercest and bitterest struggle in the Ukraine, in the very heart of which lived Winogradsky. The situation here became catastrophic. Life became worthless. Man was man's fiercest enemy. One government followed another: first the Ukrainian Getmans; then all sorts of armed bands, who sought, under the guise of the abolition of monarchy to destroy all they could; then the Germans, the Poles, the White Armies, the Red Armies, until the city of Kiev in the heart of the Ukraine had passed through more than 20 different governments within two years. Finally, all was quiet. The Revolution had won. Those that had lost the struggle ran for their lives, through Poland, through Turkey, through Siberia, wherever a way was still open. Many of the old and rich Russian families, especially those living in the South, were forced to leave Russia with the remnants of some of the old armies that retired

southward, through the Black Sea and Constantinople, and became dispersed later through Jugoslavia and Czechoslovakia, Germany, and France. Among them was Winogradsky. On board a French warship, he left Odessa and in due time arrived in Marseilles. His family was completely dispersed. His wife was left in Kiev, from which she later escaped secretly by traveling in a peasant's cart, for a distance of 200 kilometers, to the Polish boundary. Two of his daughters rejoined Winogradsky and their mother several years later.

In 1921 Winogradsky arrived, alone, at his villa in Switzerland. He brought no material riches with him. Fortunately, his greatest riches were within himself, recalling the words of the Roman philosopher, "omnia mea mecum porto." Throughout the years of his retirement, his scientific mind had not ceased to function. In one of his letters to Omeliansky, he wrote that, throughout this period, he led "la vie latente d'un savant."

The question now was not whether he was going to come back to science, but how. Since many Russian professors became established in Jugoslavia and he had some friends in Belgrade, he went first to that city, hoping to find something to do there. He was immediately appointed to a professorship at the agricultural institute of the university. This institution offered no facilities whatsoever for scientific work; there was no laboratory to speak of and less equipment. Everything had to be built up from the beginning, with very limited means. The main problem, that of obtaining support from the government or from the university, was never settled. He was asked to deliver a course of lectures, either in Russian or in French. Unfortunately, nothing was available for teaching purposes, except an empty auditorium; not even an adequate library where he could find scientific journals and books. He could not but refuse to lecture under these conditions.

Fortunately, he discovered in the director's office a complete set of the Second Abteilung of the *Centralblatt für Bakteriologie*—its presence there was never explained. This gave him an opportunity to review the field of general microbiology and of soil bacteriology in particular, and to determine the progress made during the interim of nearly seventeen years. Although progress had been made in a number of fields, very little had been done to advance knowledge of the autotrophic bacteria. Furthermore, he discovered that some of his own work, especially that on the iron bacteria, had been misunderstood and grossly misinterpreted by many workers. This led him to prepare a paper entitled "Eisenbakterien als Anorgoxydanten," which appeared in 1922 in the *Centralblatt*. It is interesting to note that after the paper was written, he did not have the facilities of a typist to copy the manuscript; his wife, who meanwhile had joined him, copied it in longhand. This paper on the iron bacteria was soon followed by a shorter note on the nitrifying bacteria, published in the *Comptes Rendus*, in which he took issue with Beijerinck's misdirected attack against him, made nearly ten years previously.

In February, 1922, Winogradsky received a letter from Dr. E. Roux, director of the Pasteur Institute, inviting him to come to Paris and organize a division of agricultural bacteriology at the institute. The invitation of Pasteur, made just

thirty years previously through Metchnikov, was now repeated. Although Winogradsky preferred in 1892 to return to St. Petersburg, the doors of the Pasteur Institute were opened again to him, even after the death of Pasteur. The second invitation, by Pasteur's student, collaborator, and successor, was accepted. Roux wrote in his letter:

My colleagues and I will be very grateful to you if you will come and establish yourself at the Pasteur Institute. You will bring to it your scientific fame and you will be able to pursue there, without being troubled by teaching duties, your magnificent investigations. After Metchnikov, we shall be proud to number Winogradsky also among our own. You will be our leader in everything that concerns the bacteriology of the soil.

This invitation fitted well the nature and needs of Winogradsky. Here he could be in contact with some of the finest minds in the field of microbiology. He did not have to live in the city of Paris itself, which he could never have tolerated for long, because of his dislike for city life in general. A small estate of 5 hectares, situated at Brie-Comte-Robert, 30 kilometers from Paris and donated to the institute, was placed at his disposal. This estate contained a large house, as well as a smaller house, greenhouses, and stables. It also had an orchard, a park, large flower beds, and some cultivated land. It thus offered facilities for the study of bacteria in soils of various states of cultivation, as well as an ideal combination for both laboratory investigation and practical field work, which agreed well with Winogradsky's temperament. Winogradsky and his family settled in the large house, while the smaller building was equipped as a laboratory, the lower floor for chemical work and the upper for microscopic work.

The outside world, from which Russia had been cut off during the period of the Civil War, was unaware of Winogradsky's fate, especially because of his retirement to private life many years previously. As soon as it was discovered that he was still alive and that he was returning to scientific work, honors were showered upon him. The French Academy of Sciences elected him a foreign associate, the English Royal Society a foreign member, and in 1925, the All Union Academy of Sciences of Soviet Russia, disregarding all political affiliations and recognizing in him a great scientific mind, elected him to honorary membership.

#### *The active Brie-Comte-Robert period (1922-1940)*

It was quite natural that upon returning to scientific work, after an interruption of many years, Winogradsky should submit to a critical review the progress that had been made during this period in his particular field of soil microbiology. This took the form of several review papers. The first two, referred to previously, had a bearing upon his own work; in these he refuted the accumulated misconceptions concerning two groups of organisms with which his name had become closely connected, the iron bacteria and the nitrifying bacteria. These were soon followed by several other papers, in which he summarized critically the various methods commonly employed in soil microbiology; he also presented an outline of other methods which he planned to utilize in his own investigations.

He began his first paper on the iron bacteria as inorgoxydants, as follows:

Since I established 35 years ago, in an early contribution, the principles of the physiology of a group of organisms which I have designated as iron bacteria, the problem has progressed only very slowly. That contribution was brief, but it offered a basis broad enough for future investigations. Although I did not succeed in undertaking these myself, or have them undertaken, my own subsequent studies of the organisms of nitrification threw so much light upon the whole group of inorgoxydants, that the existence of a broad analogy between the members of this group—the sulfur bacteria and the nitrite- and nitrate-forming bacteria, on the one hand, and the iron bacteria, on the other—could hardly remain in doubt.

He summarized the principles of nutrition of these organisms, brought out in his early work, and added:

The program for the future development of the whole problem seemed to have been laid down, but in vain; nobody followed it. The further investigations, based on errors, accumulated considerable misunderstanding, which finally led to an unbelievable confusion. . . . The corresponding chapter of bacteriology remained in a state of chaos. On this basis, I felt obliged to return to this subject, in order to make an attempt to present a more exact view of the problems involved and to further it by precise examination.

He criticized severely the work of Molisch, Löhnis, Ellis, and others, who not only discarded his ideas but confused them considerably, and who spoke of them as "Winogradsky's hypothesis," whereby the conception of the specific autotrophic physiology of these organisms was forgotten and a predominant mechanical deposition of iron by the so-called "iron bacteria" was assumed. This resulted in the confusion between "iron accumulation" and "iron secretion" by bacteria. No differentiation was made between the phenomena of precipitation of iron, as a result either of the indirect action of various organisms or of the physicochemical conditions, and the formation of an iron precipitate as a result of the specific metabolism of certain bacteria. Only the latter are to be considered as true "iron bacteria."

In the second paper, he took exception to Beijerinck's claim that a nitrifying organism can change into a physiological species which no longer possesses the capacity for nitrification. This idea of a hereditary stable modification of an organism brought about only in a medium containing traces of organic matter could hardly appeal to Winogradsky, who knew the specific physiology of the nitrifying bacteria. He concluded with a certain degree of sarcasm:

One hesitates certainly to attribute to the eminent Dutch bacteriologist, to whom science owes so many beautiful discoveries, a faulty technique. It can be rather explained by his original manner of treating the subject in question as virgin ground, paying so little attention to what others have already accomplished. M. Beijerinck does not attach any importance to the method of control of the purity of the culture established by us.

The first subject for investigation which Winogradsky undertook at the new laboratory was the direct microscopic examination of the soil. In introducing this subject, he reviewed the accomplishments so far attained in our understanding of the soil microbiological population. The reasons for doing so are best expressed in his own words:

Before a new stage [in the development of a science], there is a general tendency to weigh the acquired facts, to refresh our ideas of combating the illusions which, in spite of us, become implanted in our minds,—all this in order to select wisely the direction of efforts and the program of work. Under the influence of this tendency, the idea occurred to me to trace a summary of the actual state of our knowledge of the microbiology of the soil, a summary very sincere, born in the retirement of the laboratory, without any attempt to popularize the subject by arousing public interest. This done, I shall try to indicate, in broad outline, how, in my opinion, it would be necessary to proceed in order to make progress in a field which has remained, and not for a short time at that, in a quasi-stationary state.

He emphasized the fact that no books had been written which would give an exhaustive and critical interpretation of the subject of soil microbiology. The *Handbuch* and *Vorlesungen* of Löhnis were considered as bibliographic compilations in which no attempt was made at sufficient and clear interpretation. The books of Kayser were looked upon as mere applications of facts obtained in general microbiology to the processes taking place in the soil.

Winogradsky reviewed in detail the elective culture method which had been used so extensively in bacteriology and which had led to the isolation of a great variety of different microorganisms known to be active in certain important soil processes. Many of these organisms were considered as hothouse varieties rather than representatives of the important groups active in the soil itself. He concluded that, although general microbiology had made considerable progress in developing a better understanding of the physiology of microorganisms and their role in numerous natural processes, soil microbiology had not kept pace with this progress. It had not developed as an independent science with its own special methods. As Winogradsky pointed out, the soil is a complicated microbiological medium, and the study of this complex could not be based solely upon the isolation of a certain number of organisms in pure culture. It must be studied as a whole and in the soil itself or by methods which approach this natural medium. He recognized that the microbiology of the soil represents certain inherent difficulties not encountered in other branches of microbiology. The soil is but slightly accessible to the microscope; it is variable in chemical composition and is a poor culture medium in comparison with various artificial media; it cannot be sterilized without being materially modified. The study of microorganisms in such a natural substrate represents insurmountable difficulties. The broad and commonly accepted conclusions that the activities of the different microbes isolated from the soil, as measured upon artificial culture media, are the same as in the undisturbed soil, could, therefore, hardly be justified.

When organisms are removed from the soil and placed under optimum conditions of culture, changes are brought about in their physiology, which may not be characteristic of their activities in the soil itself. Under natural conditions, where each organism has to struggle continuously against thousands of others, it attains a certain degree of specialization which it may not possess in pure culture. At best, results obtained from general microbiology can give only analogies and hypotheses which must be verified upon the soil as the medium for microbial growth.

The introduction of the Remy-Löhnis solution method into soil microbiology, a method which together with its various modifications dominated the field for a decade and a half, had failed, Winogradsky asserted, to advance the subject very far. On adding suspensions of soil to culture solutions containing specific substances and determining the nature of the transformations taking place under these conditions, results are obtained which differ considerably from what one would expect to take place in the soil. This is due to the fact that purely artificial conditions are created, and the results are therefore of a very uncertain nature.

The true interpretation of the nature of the microbiological population of the soil in its natural condition can be made only by the direct method of investigation. The difficulties involved in the microscopic examination of the soil and the great heterogeneity of the soil population are the major stumbling blocks in any attempt to develop such methods. When a certain organic compound is added to the soil or when the physical or chemical condition of the soil is modified otherwise, the microbiological equilibrium is upset, and those species which are favored by the particular substance or treatment are able to multiply very extensively. Certain types of microorganisms are thus found to be the agents responsible for the specific transformations that take place in the soil. Winogradsky suggested, therefore, that the study of the direct modification of the soil population be supplemented by auxiliary cultures, that is, by silica gel plates containing the same substance and inoculated with particles of the same soil.

These two methods, Winogradsky pointed out, throw light upon the *microbiological state* of the soil and its *biological reaction*. The first represents the quantity of active cells of microorganisms present in the soil, and the second measures the specific modifications of the soil population resulting from the addition of the particular substance or from the particular treatment. It is not the nature of the cells which determines their role in the soil; it is rather their competition with other cells, as modified by their specific capacity to utilize the available energy sources and other nutrients and to adapt themselves to the particular conditions, that controls the microbiological activities in the soil.

These considerations led to the important conclusion that the soil, having been for ages a reservoir of all sorts of plant and animal debris, is a medium which harbors all sorts of different microbes. The great majority of these are, at any given moment, in a dormant state. The microbiological processes in the soil consist of a number of phases superimposed one upon another, each phase being dependent upon the action of a single agent or of a group of microbial agents. Some of these phases are only transitory, but they must be studied if one is to understand the microbiological processes that take place in the soil. One can thus distinguish between static and dynamic microbiology. It is only by taking all these phases into consideration, Winogradsky asserted, that one is justified in speaking of soil microbiology as a definite science.

This general outline of the broad principles underlying the study of soil microorganisms was followed by a series of papers dealing with the methods of

studying the microbiological population of the soil and with the nature of that population. As a basis for the microscopic methods, Winogradsky utilized one proposed eight years previously by the American bacteriologist H. J. Conn. This method involves fixing a dilute suspension of soil upon a glass slide, by means of agar or gelatin, then staining it with acid dyes. By this method, Conn demonstrated that the soil contains a far larger number of bacteria than can ever be obtained by plating procedures. Most of the bacteria thus found in the soil are nonspore-formers, whereas the spore-forming organisms are much less abundant and appear only under certain conditions. Winogradsky modified this method and utilized it for studying the bacterial population of the soil both in a natural state and after it had been modified by treatment, such as the addition of various organic and inorganic substances and change in moisture content and reaction. The characteristic bacterial flora which can always be found in undisturbed soil was designated as *microflore autochtone*, or native microflora.

These investigations led Winogradsky, in a paper before the First Microbiological Congress in Paris, to define the soil microbiological population as "an organized collective which always responds to a maximum degree to stimulants and to additions of available energy brought in from outside, the maximum being due—here as well as elsewhere—to the principle of division of labor, which finds its expression in the special molecular affinities or aptitudes of the members of the collective."

The first series of investigations (1923-1925) dealing with the direct microscopic examination of the soil was soon followed by a study of the organisms concerned in nonsymbiotic nitrogen fixation. The particular capacity of the soil or of the organisms inhabiting it to fix atmospheric nitrogen was measured either by adding a source of available energy to the soil or by inoculating the soil into a liquid or solid medium containing a source of energy but very little combined nitrogen. Although in his early work on the nitrogen-fixing bacteria Winogradsky himself employed liquid cultures, he could not approve of them for his later work, when considerable information had accumulated concerning the members of the soil population capable of bringing about nitrogen fixation. Liquid cultures offered the disadvantage that the most active nitrogen-fixing Azotobacter is not favored in a medium which is poorly aerated and which becomes rapidly contaminated with a number of other organisms, especially the butyric-acid bacteria. On the other hand, as a medium, the soil has the disadvantage that only a small part can be used for analysis, as a result of which a small error multiplied many times becomes significant. Winogradsky suggested the use of the silica gel plate containing a definite amount of mannitol or other carbonaceous substance as a source of energy and inoculated with a small quantity of soil. The number of Azotobacter colonies developing on the plate within two or three days can serve as a measure of the abundance of these organisms in the soil. The amount of nitrogen fixed in the plate after nine or ten days can be used as a measure of the nitrogen-fixing capacity of the given soil.

Winogradsky further proposed another method, which consists in sieving the

soil and adding 5 per cent pulverized starch and water to make a thick paste; this paste is then packed into small Petri dishes, 5 cm. in diameter. The surface of the soil is polished with a glass slide moistened with water, and the dishes are incubated at 30°C. If Azotobacter is present, typical moist colonies will appear, after 48 hours, in a nearly pure state covering the surface of the soil. This method, which came to be known as the "soil plaque method," found extensive application in numerous practical studies on phosphate deficiency in soil. It may be considered as a modification of the spontaneous culture method used extensively by Winogradsky for establishing the role of certain microorganisms in specific soil processes.

By means of these methods, all soils were divided into four groups, on the basis of their nitrogen-fixing capacity, as follows: very active, not very active, temporarily inactive, and permanently inactive. In a series of papers published during 1926-1928, Winogradsky summarized the current status of the subject of nitrogen-fixing organisms in the soil, the methods of studying their occurrence and abundance, the conditions favoring their activities, and their importance in soil processes. Numerous investigations had been made of the occurrence and abundance of nitrogen-fixing bacteria in the soil, and since Winogradsky's first contribution in 1893, nearly a thousand papers had been published dealing with this subject. Still, with the exception of H. Christensen in Denmark and others soon following who utilized Azotobacter for determining phosphorus deficiency in soil, no one had made any practical use of this information. The importance of these organisms in soil processes could not even be stated definitely, although numerous opinions had been expressed.

Winogradsky pointed out that in many cases it is sufficient to determine the abundance of Azotobacter in a given soil and the response of this organism to soil treatment, in order to estimate the specific fertilizer needs of the soil. He showed that the addition of nitrogenous fertilizer represses the development of Azotobacter, an organism active only in soils lacking in combined nitrogen, and that an abundance of the combined nitrogen leads to its disappearance.

The mechanism of nitrogen fixation by Azotobacter was next (1930-1932) studied. Several theories had been proposed formerly to explain this process. It is sufficient to mention Winogradsky's own ideas in connection with his early work on *Clostridium*, whereby he assumed that ammonia is the first step in the fixation of nitrogen; more recently Kostytschev claimed to have demonstrated the formation of ammonia in the process of nitrogen fixation by *Azotobacter*. By the use of newer methods of analysis and more detailed investigations, these earlier ideas were now fully confirmed. The hydrogenation of the molecular nitrogen was found not to go hand in hand with protein synthesis. Consequently, the assimilation of nitrogen by the bacteria was believed to proceed in two stages; one, fixation which results in ammonia production, and the other, the assimilation of the ammonia.

No sooner had Winogradsky progressed thus far with the problem of nitrogen fixation than he became interested in another important microbiological process, the decomposition of cellulose by bacteria. This subject, like that of nitrogen

fixation, was not new to him. In 1895, he first attacked the highly important process of bacterial decomposition of such complex carbohydrates as the pectins. Unfortunately, that problem was studied for only a short time, because his student Fribe, to whom it was assigned, dropped it without bringing it to a logical conclusion. Soon afterward, however, his other assistant, Omeliansky, took up the subject of anaerobic bacteria capable of decomposing cellulose. Although he made an important contribution to this subject, Omeliansky failed to isolate in pure culture the bacteria concerned and to establish definitely whether they are the true causative agents. This resulted in considerable confusion regarding the specific nature of the bacteria active in cellulose decomposition. Some investigators asserted that Omeliansky's conclusions were not justified and that the true agents of cellulose decomposition are aerobic bacteria, whereas the anaerobic organisms must be considered as secondary invaders. Kellerman and associates in the United States isolated certain weak cellulose-decomposing aerobic bacteria from Omeliansky's cultures, a result which they considered as evidence for these assertions.

While Omeliansky was conducting these investigations, Winogradsky had apparently shown little interest in the whole problem. During his retirement to private life, between 1905-1920, when he made his daily rounds through the forests on his estates in the Podol, he must have stopped many times to examine the organic matter accumulations in those soils. This must have led him to question the mechanism of the disappearance of the plant residues, a question unanswered in his own mind until 1928, when he decided upon a detailed study of this phenomenon.

The disappearance of cellulose in nature is now known to be brought about through the action of a large number of different organisms. Various groups of bacteria, a large number of fungi and actinomycetes, and possibly certain protozoa and other invertebrate animals are capable of decomposing cellulose. The bacteria comprise many different forms, ranging from anaerobes to aerobes, and including spore-formers and nonspore-formers. The role of the aerobic bacteria in the process attracted Winogradsky's particular attention. Although a number of aerobic bacteria capable of decomposing cellulose had been described, and some, notably *Spirochaeta cytophaga* of Hutchinson and Clayton (1918), had even been shown to be very active forms, considerable confusion still existed concerning their exact nature and identity.

Winogradsky's preliminary paper on cellulose-decomposing bacteria appeared in 1926, but the final memoir was not completed until 1929. He was not so much interested in the nature of the cellulose-decomposing microbiological population in the soil as in those organisms which play an important role in the process under natural conditions. He recognized the existence of several different genera of bacteria characteristically capable of attacking true cellulose in the soil. The mechanism of cellulose decomposition was believed to involve its oxidation to oxycellulose or to a carbohydrate acid, which is the building stone of soil humus. These bacteria were thus considered to play an important role in the production of an organic colloid in the soil. Winogradsky took

occasion, in this connection, to argue against the present writer's conception that the so-called oxycellulose is not a product of oxidation of cellulose by bacteria and is not even an oxycellulose, but is merely a product of microbial synthesis, a hemicellulose of the polyuronide type. Winogradsky suggested that though this may be true in the case of fungi as agents of cellulose decomposition, with which the work of the writer was largely concerned, the oxidation process holds in the case of aerobic bacteria. Whatever may be the true mechanism of the formation of the slimy substance in the decomposition of plant residues in soils and in composts, its importance in humus formation is generally underestimated. It contributes polyuronide compounds which are always present in humus. Winogradsky further showed that in the aerobic decomposition of cellulose by bacteria, no fatty acids or volatile substances are produced.

This investigation was immediately followed (1931-1933) by a detailed study of the agents concerned in the nitrification process. Since his first papers appeared, forty years previously, hundreds of contributions, in all parts of the world, had been made to this subject, because of its great significance in nitrogen transformations and plant nutrition. In the great majority of cases, subsequent investigators did little more than confirm the early results of Winogradsky. Only in the study of the energetics of the process was definite progress made, particularly in the work of Meyerhoff and Bonazzi. On the other hand, various papers continued to appear, in which claims were made that a great variety of bacteria, not all of which were autotrophic in nature, are capable of producing nitrite from ammonia, and nitrate from nitrite; the quantities of nitrite and nitrate produced by these organisms were usually very small, much less than those produced by the classical autotrophic bacteria of Winogradsky. Other investigations, as those of Hagem on the influence of soil reaction on the nitrification process, and of Romell on the occurrence of nitrifying bacteria in acid forest soils, confirming in the main the results of Winogradsky, still pointed to certain unexplained variations. Winogradsky considered it essential, therefore, to make a new detailed investigation of this problem and settle once and for all the true nature of the nitrifying organisms, their variation and activities.

Contrary to his early ideas that only two groups of organisms are concerned in the formation of nitrite and nitrate in nature by oxidation processes, Winogradsky now found that the soil harbors a more abundant flora of nitrifying bacteria capable of adapting themselves to natural conditions under which the process is carried out.

Three genera of bacteria capable of oxidizing ammonia to nitrite were now described: *Nitrosomonas*, comprising the original form described in 1891; *Nitroscystis*, the cyst form found in forest soils and in sewage sludge; and *Nitrosospira*, a spirochaete-like form found seldom, and then only in virgin soil. These three groups of organisms vary not only in their morphology but also in their physiology and in their speed of action. The first group has a much wider range of reaction for development than the last two, although its optimum is at pH 7.4-7.8, and for some strains even at 8.6-9.2. The last group was found

to be most active at somewhat more acid reactions. Winogradsky's daughter later added a fourth group, isolated from activated sludge, *Nitrosogloea*, represented by several species.

The nitrate-forming group of bacteria was found to comprise, in addition to the originally described *Nitrobacter*, other forms given the generic names of *Bactoderma*, growing in a membrane, and *Nitrocystis*, producing zooglea and found abundantly in activated sewage sludge. A fourth group, *Nitrogloea*, was found in the same substrate.

These investigations completed, Winogradsky turned again to the organisms concerned in the fixation of nitrogen and to the mechanism of the process. In 1938 he made a detailed study of the Azotobacter group, their occurrence in soil and in water, and their life cycle. The morphology of these organisms appeared to be particularly instructive because it throws light upon the principle of bacterial classification. In examining their life cycle, Winogradsky came to the conclusion that all the stages must be taken into consideration. These stages were manifested, however, with a certain degree of stability, only under conditions convenient to specific adaptations; if these conditions were modified, the species reacted by evolutionary stages which represented a type of pathological morphology rather than of normal morphology. He emphasized the fact that a lack of recognition of these fundamental principles frequently led to considerable confusion and to badly conceived doctrines of bacterial life cycles and cyclogenetic theories. Under purely artificial laboratory conditions, bacteria form types or stages which cannot be considered as normal but must rather be classed as mutilates or aberration forms. Winogradsky emphasized again the importance, in the case of Azotobacter, of using organisms freshly isolated from natural substrates, without the use of standard methods of enrichment cultures. The use of ethyl alcohol for the isolation of these organisms was recommended. A new genus, *Azomonas*, was created, comprising primarily water-inhabiting forms.

Winogradsky published in the same year (1938) a highly critical review of the literature on the mechanism of nitrogen fixation, with special reference to the role of ammonia as the first stage in the fixation process. He emphasized particularly the bearing that his own work had upon these investigations. He finally undertook a critical survey of the work carried out during 1887-1920 on nitrification and nitrogen fixation, with special emphasis upon ecological relationships. He emphasized particularly that general microbiology is to be distinguished from ecological microbiology, the former treating the activities of the organism in pure culture, and the latter dealing with spontaneous cultures, characterized by the free development of microorganisms in natural substrates, limited only by the competition of the other organisms comprising the natural population. He emphasized particularly the biological equilibrium attained in an undisturbed soil. This equilibrium was said to consist largely of cocci united into zooglea, which live upon the organic gel of the soil.

Fortunately, these investigations did not end the scientific activities of Winogradsky. His daughter, Helen, who had become his assistant, proceeded with the nitrification studies, while he began, with renewed vigor (1936), in-

vestigations of nitrogen fixation by leguminous plants. Here, as with the Azotobacter problem, he was interested in the mechanism of nitrogen fixation. Since the first demonstration of the role of bacteria in the formation of nodules and in the fixation of nitrogen by the leguminous plants, considerable attention had been devoted to the function of the two symbionts in the process. Beijerinck, as late as 1924, still adhered to his early ideas that the plant is the active agent in the fixation process, while the bacteria produce certain stimulants, some sort of hormone, which renders the plant capable of performing the fixation process. Winogradsky, on the other hand, assumed the bacteria to be the active agents. He claimed to have demonstrated that this process is carried out in the nodule, even when the latter is severed from the plant.

At the age of eighty-four, when the writer last saw him, Winogradsky was still vigorous and his mind was as alert as that of a youth. He was still engaged in solving some of the puzzling problems in microbiology.

#### *Period of forced retirement (1940-1946)*

With the invasion of France, Winogradsky was forced into a second period of retirement. At first, he faced the invasion in a cool spirit. This is evidenced by a letter written by him on June 9, 1940, on the eve of the invasion of Paris:

I am directing all my efforts toward carrying through the burden of all the horrible events. Meanwhile, we are not experiencing any material lack. Everything goes on as usual, except for the fact that we are just behind the fighting armies. Although complete quiet reigns in the country, we feel highly depressed and restless, especially these days when constant fighting is going on, as you know.

I try to forget myself in my work. I am writing the tenth, presumably the last, memoir of the series *Études*, etc. It is in this connection that I want to ask a favor of you. It is quite impossible for this work to appear here in a normal period of time, because of the numerous limitations imposed. In general, the future appears dark not only because of the subject, but because of the author himself.<sup>2</sup> I intend, therefore, to place the English text (condensed as much as possible) into your friendly hands and let you do with it whatever you decide. Please go over the text and correct the various lapses in expression. SOIL SCIENCE may offer the hospitality of its pages. It is out of the question to send proof here. Do not order any additional reprints, for these are too expensive and the means are not available. I expect to send you the manuscript at the end of the month. . . .

The manuscript never arrived. It was published later in the *Annales of the Pasteur Institute*. The results of this, the tenth of a series of memoirs, have been summarized by Winogradsky as follows:

1. The Azotobacter group is primarily a producer of *azohydrase*, an enzyme for ammonia synthesis; a part of the product, depending on conditions of nutrition, is immobilized by the cells, and the rest is excreted.
2. In admitting an *azohydrase* system, one must admit the bearing of the dehydrogenating action upon the nutrition of the organism.
3. The action of the enzyme continues, independent of the vital activities of the cells and resistant to direct oxidation by microorganisms, for a considerable time even after the destruction of the cells.
4. This continuous action produces quantities of ammonia far greater than those assimilated by the cells.

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<sup>2</sup> Referring to the fact that he is a Russian in France.

5. The total nitrogen retained by the cells is resistant and is not subject to any reduction during the prolonged liberation of the ammonia.

6. The production of ammonia is of economic significance, since very considerable amounts are produced in the process of consumption of limited amounts of nutrients.

Winogradsky believed these results to be of great significance in interpreting the activity of Azotobacter in soils and in water basins poor in nitrogen. He advanced the hypothesis that the same chemical process is involved in all reactions of nitrogen fixation brought about by microorganisms. He emphasized the fact that the role played by the physical and chemical processes of fixation of nitrogen in nature is quite insignificant when compared with the action of microorganisms.

#### WINOGRADSKY'S PLACE IN SCIENCE

Winogradsky is, first of all, an accurate observer. As Romell characterized him, "He combines, on the one hand, the keen eye of the genial nature observer with the experimental instinct and ability of the perfect laboratory man. He combines, on the other hand, a very pronounced ecological 'feel' with the craving for exact analysis by impeccable logic."<sup>3</sup> This combination is found very seldom among scientists. The observer rarely has the capacity for exact experimental investigations. On the other hand, the experimentalist frequently lacks the ecological outlook. Many of Winogradsky's early physiological observations, especially on the sulfur and iron bacteria, were made largely by the use of the microscope. His chemical methods were very simple. They always gave valuable results because they were invariably supplemented by careful and most painstaking observation. He has always disliked "standard methods" and usually has devised his own simple and direct methods adapted to the particular problem. This happy combination of observation and experimentation have resulted in his brilliant contributions to microbiology.

Omeliansky thus characterized the scientific personality of Winogradsky:

The scientific ideas of Winogradsky represent a series of daring attempts to penetrate fields of knowledge not yet touched. The unknown attracts him. Here his scientific touch and his ability to approach a problem simply and accurately can be well expressed. "I feel myself, in a scientific sense, at complete freedom in a field still to be investigated," he wrote in connection with one of his latest works, "and for me that is the greatest stimulus." With each work, Winogradsky has opened new fields for investigation and pointed ways for their further study. Many branches of modern microbiology can be said to have been created by the efforts of this prominent scientist, of whom Russian science may justly be proud.

His scientific argumentation possesses conviction and conquering force. One feels that long consideration has preceded the beginning of the investigation and that before he begins to "wagen", he has to "wägen" for a long time. The manner of exposition in his scientific papers is characteristic and interesting. It is always highly business-like in nature. He approaches the question from its most important angle, avoiding all unnecessary prelim-

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<sup>3</sup> In a personal note.

inaries. Without hiding behind words, he penetrates immediately *in medias res*, directly approaching the essence of the problem.

Romell<sup>4</sup> has noted a great similarity between De Saussure and Winogradsky. The style of both is one of elegant simplicity and pleasant clarity. Sach's reference to De Saussure applies to both: "Er verstand es die Fragen an die Natur so zu stellen, dass die Antwort notwendig klar werden musste." The work of both was largely obscured for a number of decades by numerous inferior contributions of others. Romell has pointed out that the difference between the two is that De Saussure's work was revived by Liebig, whereas Winogradsky has lived to revive his own work.

Winogradsky has attacked some of the most difficult problems in the field of microbiology, and his name will be forever connected, in particular, with certain phases of this young but rapidly growing science. His investigations have dealt primarily with microorganisms of a highly specific physiology, which sets them apart from other microbes. These organisms are established as important agents in the cycle of life in nature—transforming such elements as nitrogen, sulfur, and iron in the soil, in the sea, and in sewage—as well as in a great variety of processes upon which the very life of plants and animals is based. Winogradsky is one of the few true pathfinders in microbiology. He is in the true sense a *microbe hunter*, a hunter not for injurious microbes, but for beneficial organisms, the best friends that man has in his struggle for existence. Among the various groups of bacteria studied by Winogradsky and with which his name has become primarily associated, none is more important and more specific than the group of autotrophic bacteria, with which he began his epoch-making work in bacteriology and which have served as the subject for some of his more recent studies.

The name of Winogradsky has assumed a permanent place in bacteriology through the profound influence of his investigations upon the subsequent development of many important branches of the science. As a result of the brilliant and epoch-making investigations of Louis Pasteur, Ferdinand Cohn, and Robert Koch, bacteriology developed rapidly from a mere biological curiosity into a science of great practical importance, with numerous ramifications, stretching into the domains of medicine, agriculture, industry, and certain arts. Winogradsky's name is particularly associated with certain special branches of bacteriology, which he not only discovered but has also developed to a high stage of perfection. These include, besides his studies on the autotrophic bacteria, his contributions to knowledge of the nonsymbiotic nitrogen-fixing bacteria, as well as his investigation on the oxidation of sulfur, the bacteriology of cellulose decomposition, and the microbiology of the soil. His work thus has proved to be the basis for a better understanding of the nature and physiology of various highly specialized and important groups of bacteria.

Not only the microbiology of the soil, but general microbiology and general physiology as well, will always be more complete, more truly fundamental, and more sound because of the work of Sergei Nikolaevitch Winogradsky.

<sup>4</sup> Personal communication.

LIST OF PUBLICATIONS  
of  
S. N. WINOGRADSKY

- Ueber die Wirkung äusserer Einflüsse auf die Entwicklung von *Mycoderma vini*. *Pubs. St. Petersburg Soc. Nat. Sci.* 14 (pt. 2): 132-135. 1884. (German abstract in *Bot. Centbl.* 20: 165-167. 1884).
- Ueber Schwefelbakterien. *Bot. Ztg.* 45: 489-507, 513-523, 529-539, 545-559, 569-576, 585-594, 606-610. 1887.
- Ueber Eisenbakterien. *Bot. Ztg.* 46: 261-270. 1888.
- Beiträge zur Morphologie und Physiologie der Bakterien: I. Zur Morphologie und Physiologie der Schwefelbakterien. Arthur Felix, Leipzig. 1888.
- Recherches physiologiques sur les sulfobactéries. *Ann. Inst. Pasteur* 3: 49-60. 1889.
- Sur le pléomorphisme des bactéries. *Ann. Inst. Pasteur* 3: 249-267. 1889.
- Sur les organismes de la nitrification. *Compte. Rend. Acad. Sci. [Paris]* 110: 1013-1016. 1890.
- Sur la formation et l'oxydation des nitrites pendant la nitrification. *Compt. Rend. Acad. Sci. [Paris]* 113: 89-92. 1891.
- Recherches sur les organismes de la nitrification. *Ann. Inst. Pasteur* 4: 213-231, 257-275, 760-771. 1890; 5: 92-100, 577-616. 1891.
- Ueber die Organismen der Nitrification. Vortrag gehalten in der Sitzung der Zurich naturforschenden Gesellschaft, 22 Februar, 1891.
- Contributions à la morphologie des organismes de la nitrification. *Arch. Sci. Biol. [St. Petersburg]* 1: 87-137. 1892.
- Sur l'assimilation de l'azote gazeux de l'atmosphère par les microbes. *Compt. Rend. Acad. Sci. [Paris]* 116: 1385-1388. 1893; 118: 353-355. 1894.
- Sur le rouissage du lin et son agent microbien. *Compt. Rend. Acad. Sci. [Paris]* 121: 742-745. 1895.
- Sur l'assimilation de l'azote libre de l'atmosphère par les microbes. *Arch. Sci. Biol. [St. Petersburg]* 3: 297-352. 1895.
- Zur Mikrobiologie des Nitrifikationsprozesses. *Centbl. Bakt. (II)* 2: 415-428, 449-458. 1896.
- On the role of microbes in the general cycle of life. (In Russian.) Address delivered before the Imperial Institut of Experimental Medicine, December 8, 1896. St. Petersburg. 1897. (Other reports and addresses delivered in Russian either were not published or were lost in the archives of the Society for Natural Science and of various conferences.)
- L'influence des substances organiques sur le travail des microbes nitrificateurs. (With V. Omeliansky.) *Arch. Sci. Biol. [St. Petersburg]* 7: 233-271. 1899.
- Clostridium Pastorianum*, seine Morphologie und seine Eigenschaften als Buttersäureferment. *Centbl. Bakt. (II)* 9: 43-54, 107-112. 1902.
- Die Nitrifikation. Lafar's *Handb. Tech. Mykol.* 3: 132-181. 1904-1906.
- Eisenbakterien als Anorgoxydanten. *Centbl. Bakt. (II)* 57: 1-21. 1922.
- Sur la prétenue transformation du ferment nitrique en espèce saprophyte. *Compt. Rend. Acad. Sci. [Paris]* 175: 301-304. 1922.
- Sur la méthode directe dans l'étude microbiologique du sol. *Compt. Rend. Acad. Sci. [Paris]* 177: 1001-1004. 1923.
- La méthode directe dans l'étude microbiologique du sol. *Chim. et Indus. [Paris]* 11: (2) 1-8. 1924.
- Sur la microflore autochtone de la terre arable. *Compt. Rend. Acad. Sci. [Paris]* 178: 1236-1239. 1924.
- Sur l'étude microscopique du sol. *Compt. Rend. Acad. Sci. [Paris]* 179: 367-371. 1924.
- Sur l'étude de l'anaérobiose dans la terre arable. *Compt. Rend. Acad. Sci. [Paris]* 179: 861-863. 1924.

- Sur une méthode pour apprécier le pouvoir fixateur de l'azote dans les terres. *Compt. Rend. Acad. Sci. [Paris]* 180: 711-716. 1925.
- Études sur la microbiologie due sol: I. Sur la méthode. *Ann. Inst. Pasteur* 39: 299-354. 1925.
- Sur le pouvoir fixateur des terres. *Compt. Rend. Acad. Sci. [Paris]* 182: 907-910. 1926.
- Sur les cultures spontanées des microbes fixateurs. *Compt. Rend. Acad. Sci. [Paris]* 182: 999-1001. 1926.
- Études sur la microbiologie du sol: II. Sur les microbes fixateurs d'azote. *Ann. Inst. Pasteur* 40: 455-520. 1926.
- Sur le diagnostic de l'aptitude du sol à fixer l'azote. *Compt. Rend. Acad. Sci. [Paris]* 182: 1061-1063. 1926.
- Sur la décomposition de la cellulose dans le sol. *Compt. Rend. Acad. Sci. [Paris]* 183: 691-694. 1926.
- Recherches sur la dégradation de la cellulose dans le sol. *Compt. Rend. Acad. Sci. [Paris]* 184: 493-496. 1927.
- Sur le pouvoir fixateur des terres. *Compt. Rend. Cong. Chim. Indus.* 7: 1-10. 1927.
- Principes de microbiologie du sol. *Ann. Inst. Pasteur* 41: 1126-1138. 1927.
- The direct method in soil microbiology and its application to the study of nitrogen fixation. *Soil Sci.* 25: 37-43. 1928.
- Études sur la microbiologie due sol: III. Sur le pouvoir fixateur des terres. *Ann. Inst. Pasteur* 42: 36-62. 1928.
- Sur l'oxydation de la cellulose dans le sol. *Compt. Rend. Acad. Sci. [Paris]* 187: 326-329. 1928.
- Études sur la microbiologie du sol: IV. Sur la dégradation de la cellulose dans le sol. *Ann. Inst. Pasteur* 43: 549-633. 1929.
- Sur la synthèse de l'ammoniac par les Azotobacter du sol. *Compt. Rend. Acad. Sci. [Paris]* 190: 661-664. 1930.
- L'état actuel du problème de la fixation de l'azote atmosphérique et ses récents progrès. *Acad. Agr. France.* May 14, 1930: 1-7. 1930.
- Analyse microbiologique du sol. *Cong. Internat. Microbiol.* 1: 1-12. 1930.
- Nouvelles recherches sur les microbes de la nitrification. *Compt. Rend. Acad. Sci. [Paris]* 192: 1000-1003. 1931.
- Les agents microbiens de la nitrification. Recherches nouvelles. *Acad. Agr. France* May 20, 1931: 1-9. 1931.
- Études sur la microbiologie du sol: V. Analyse microbiologique du sol, principes d'une nouvelle méthode. *Ann. Inst. Pasteur* 48: p-89-134. 1932.
- Études sur la microbiologie du sol: VI. Sur la synthèse de l'ammoniaque par les azotobactères du sol. *Ann. Inst. Pasteur* 48: 269-300. 1932.
- Études sur la microbiologie du sol: VII. Nouvelles recherches sur les organismes de la nitrification. (Avec Hélène Winogradsky.) *Ann. Inst. Pasteur* 50: 350-433. 1933.
- Sur le dégagement de l'ammoniac par les nodosités des racines des Legumineuses. *Compt. Rend. Acad. Sci. [Paris]* 197: 209-212. 1934.
- The method in soil microbiology as illustrated by studies on Azotobacter and the nitrifying organisms. *Soil Sci.* 40: 59-76. 1935.
- Études sur la microbiologie du sol: VIII. Recherches sur la bactéries radicicoles des légumineuses. (Avec Hélène Winogradsky.) *Ann. Inst. Pasteur* 56: 221-250. 1936.
- The doctrine of pleomorphism in bacteriology. *Soil Sci.* 43: 327-340. 1937.
- Sur l'origine de l'ammoniac dégagée par les fixateurs d'azote. *Centbl. Bakt. (II)* 97: 399-413. 1938.
- Études sur la microbiologie du sol and des eaux: IX. Sur la morphologie et l'écologie des Azotobacter. *Ann. Inst. Pasteur* 60: 351-430. 1938.

- La microbiologie oecologique. *Cong. Microbiol. Langue Franç.* 1: 1-25. 1938.  
Sur la synthèse biogène de l'ammoniac dans le sol et les eaux. *Trans. 3rd Comm. Internat. Soc. Soil Sci.* B: 37-39. 1939.  
Études sur la microbiologie du sol: X. Sur la synthèse enzymatiques de l'ammoniac dans le sol et les eaux. *Ann. Inst. Pasteur* 66: 97-128. 1941.  
A series of reviews in the field of soil microbiology. *Bul. Inst. Pasteur* 25: 289. 1927;  
26: 625. 1928 (iron bacteria); 28: 681. 1930 (nitrifying bacteria); 30: 971. 1932;  
31: 945. 1933; 32: 1153. 1934; 33: 110, 310. 1935 (nitrification).

# VARIATIONS IN YIELD OF WHEAT AS AFFECTED BY DEPTH OF SOIL<sup>1</sup>

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The purpose of the work reported here was to compare, under controlled conditions, the yield of wheat grown on soils of different depths.

## MATERIALS AND METHODS

The principal soil types occurring in eastern Szechuan were used in this experiment. They are Chungking clay loam (a young neutral purple soil), Kialingkiang very fine sandy loam (a young calcareous soil), and Tsinyunszu sandy clay loam (a podzolic yellow earth).<sup>3</sup> To correspond with prevailing farming conditions, the surface soil of the first two types and the subsurface soil (B horizon) of the third were selected for crop growth. The B horizon of Tsinyunszu sandy clay loam was chosen because the surface of this soil type is usually lost by erosion following deforestation and cultivation, and thus farming is restricted to the exposed subsurface soil.

Small brick-walled tanks, each having a surface area of  $\frac{1}{2}$  square meter, equal to 1 square Chinese foot, were used as soil containers. They were provided with bottoms which allowed free drainage and which were placed at levels corresponding to the designated depths of the soils. The experiment was laid out at Pehpei, Szechuan, in 1942 in the design of a 3 by 8 split-plot randomized block.<sup>4</sup> The three soil types were considered to be the main treatments, and eight soil depths—10, 15, 20, 30, 40, 50, 70, and 100 cm.—the subtreatments. The construction and arrangement of the tanks are shown in figure 1. The tanks were filled with air-dried soil, and water was then added to bring the moisture content of the soil in each tank approximately to its moisture-holding capacity. No water other than rainfall was allowed to alter the moisture contents of the tanks during the growing period. In early winter of 1942, seeds were planted at the rate of four per hill and 25 hills per plot. Subsequently, the plants were thinned to two per hill. Liquid night soil was applied as a fertilizer at the rate of 100 cc. per plot at the time of planting and again 8 weeks later. The grain was oven-

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<sup>2</sup> Soil technologist. The author takes this opportunity to thank H. Yu for helpful suggestions in carrying out the work, and K. C. Hou and C. K. Lee for encouragement and assistance in the preparation of the manuscript.

<sup>3</sup> Yu, H., and Lee, C. K. 1944 Soils of Szechuan Province. Natl. Geol. Survey China, Soil Bul. 24. [Condensed from a complete report in Chinese.]

<sup>4</sup> Goulden, C. H. 1939 Methods of Statistical Analysis. John Wiley & Sons, Inc., New York.

dried soon after harvest and then weighed. The experiment was repeated in 1943, but no fertilizer was added.

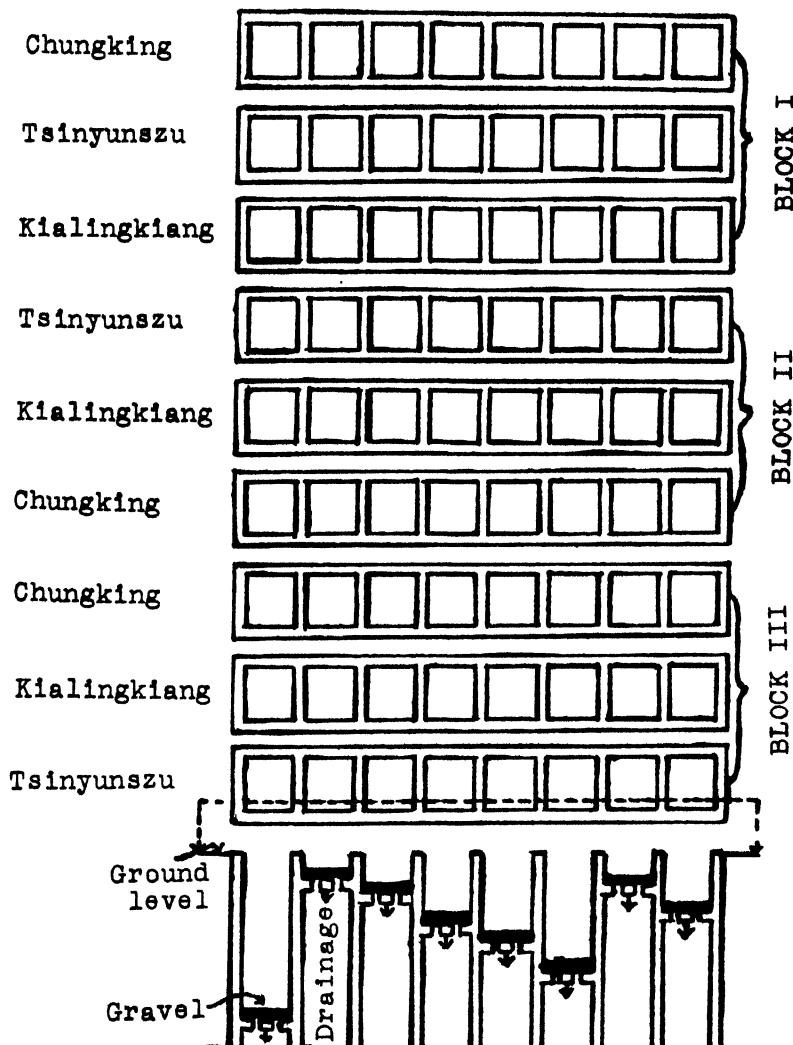


FIG. 1. CONSTRUCTION AND ARRANGEMENT OF THE EXPERIMENTAL TANKS

#### RESULTS AND DISCUSSION

Table 1 presents the results of the experiment, and table 2 the analysis of variance of the data. From the analysis of variance, it is clear that the effects of the individual factors and interactions were all highly significant. Table 1 shows that the average yields of the first harvest were significantly higher than those of the second. This may be attributed to the beneficial effects of fertilizer and to the more favorable weather conditions for the first harvest. Table 3

shows that the rainfall was lower and the evaporation higher during the growing season for wheat in the second harvest year than in the first. On these grounds, water shortage, in addition to the deficiency of plant nutrients, might be considered an important factor limiting the growth of the second crop.

TABLE 1  
*Yields of wheat on Szechuan soils of different depths in 3 by 8 split-plot randomized block experiment*  
 Results in grams of over-dried grain per plot

DEPTH OF SOLUM cm.	CHUNGKING CLAY LOAM				KIALINGKUANG VERY FINE SANDY LOAM				TSINYUNSU SANDY LOAM				AVERAGE
	Block I	Block II	Block III	Average	Block I	Block II	Block III	Average	Block I	Block II	Block III	Average	
<b>1943-43</b>													
10	32.00	43.37	48.70	41.36	43.36	58.78	48.03	50.06	3.02	2.23	4.70	3.32	31.58
15	40.35	69.63	55.75	55.24	43.57	50.63	52.67	48.87	8.30	23.00	6.40	12.57	38.89
20	50.35	51.75	52.80	51.63	55.52	60.26	65.70	60.49	9.34	18.05	11.50	12.96	41.70
30	52.65	56.55	53.85	54.35	72.50	72.75	70.87	72.04	7.85	20.80	13.75	14.13	46.84
40	48.12	67.24	69.72	61.56	56.43	69.50	63.72	63.22	14.10	22.06	22.00	19.39	48.05
50	49.47	61.87	58.70	56.68	66.85	74.50	75.06	72.16	9.03	15.00	22.85	15.63	48.15
70	43.35	74.28	60.65	59.43	74.09	81.15	97.20	84.15	20.52	31.05	21.53	24.37	55.98
100	56.35	92.86	79.00	76.07	74.86	92.15	87.07	84.69	16.73	13.36	30.73	20.27	60.35
Mean. ....	46.58	64.69	59.85	57.04	60.90	69.94	70.04	66.96	11.11	18.19	16.68	15.33	46.44
<b>1943-44</b>													
10	5.41	9.66	14.60	9.89	9.40	12.60	10.41	10.80	2.96	9.33	5.68	5.99	8.89
15	7.20	13.60	14.33	11.71	5.52	9.11	9.30	7.98	9.60	3.03	5.31	5.98	8.56
20	5.95	9.88	17.90	11.24	9.55	8.99	15.32	11.29	3.88	4.67	5.54	4.70	9.08
30	18.50	29.30	24.30	24.03	24.44	11.20	14.80	16.81	3.81	9.40	8.90	7.37	16.07
40	21.11	29.00	19.31	23.14	20.55	19.10	19.44	19.70	5.14	13.31	8.00	8.82	17.22
50	30.22	31.71	31.42	31.12	32.80	26.70	19.81	26.44	13.00	11.40	9.39	11.26	22.94
70	53.20	56.90	52.60	54.23	29.22	40.85	47.90	39.32	14.44	14.11	12.30	13.62	35.72
100	63.50	81.90	62.50	69.30	64.65	69.99	88.98	74.54	19.01	14.40	13.00	15.47	53.10
Mean. ....	25.64	32.74	29.62	29.33	24.52	24.82	25.75	25.86	8.98	9.96	8.52	9.15	21.45
2-year average....	36.11	48.72	44.73	43.19	42.71	47.38	49.14	46.41	10.05	14.08	12.60	12.24	33.95

Of the three soil types, the subsurface layer of the podzolic yellow earth is the least fertile for wheat growth. This conclusion is in accord with previous field observations.<sup>5</sup> For corresponding soil depths the two young soils produced virtually the same average yields for the 2-year period, although in the first year the alluvial soil appeared more productive than the purple soil.

<sup>5</sup> Hou, K. C. 1940 A preliminary study of the soils of Chungking area, Szechuan. Natl. Geol. Survey China, Soils Quart., vol. 1, no. 3. [In Chinese.]

TABLE 2  
*Analysis of variance of experimental data*

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN VARIANCE	F	F-POINT		SE.d
					5 per cent	1 per cent	
Block	2	1,456.5311	728.2656	8.08	6.94	18.00	1.93
Soil type	2	34,169.6113	17,084.8057	189.57	6.94	18.00	1.93
Error (a)	4	360.5017	90.1254				
Main plot	8	35,986.6441					
Solum depth	7	18,662.9385	2,666.1343	90.56	2.24	3.10	1.80
Soil type $\times$ depth	14	4,383.6404	313.1172	10.64	1.94	2.50	3.12
Error (b)	42	1,236.5272	29.4411				
Subplot	71	60,269.7502					
Year	1	22,490.7510	22,490.7510	316.20	4.04	7.19	1.30
Year $\times$ soil type	2	7,449.6079	3,724.8040	52.37	3.19	5.18	2.42
Year $\times$ depth	7	2,236.6201	319.5172	4.49	2.21	3.04	3.96
Year $\times$ soil type $\times$ depth	14	2,099.8805	149.9915	2.11	1.90	2.48	6.87
Error (c)	48	3,414.1103	71.1273				
Total	143	97,960.7200					

TABLE 3

*Monthly precipitation and evaporation at Pehpei, Szechuan, during the growing season\**

MONTH	1942-43	1943-44	DIFFERENCE
	mm	mm	mm
<i>Precipitation</i>			
November	72.3	32.6	39.7
December	21.3	9.5	11.8
January	7.6	6.8	0.8
February	23.3	11.3	12.0
March	35.8	36.6	-0.8
April	77.0	45.4	31.6
Total	237.3	142.2	95.1
<i>Evaporation</i>			
November	19.7	29.5	-9.8
December	15.0	24.2	-9.2
January	19.7	24.6	-4.9
February	23.4	43.1	-19.7
March	38.0	57.5	-19.5
April	39.9	66.6	-26.7
Total	155.7	245.5	-89.8

\* Data furnished by the National Research Institute of Meteorology, National Academia Sinica.

The deeper soils produced more grain than the shallower ones. This relationship, however, was not so pronounced in the podzolic yellow earth, probably because of the infertility of the entire solum. In the alluvial soil, the effect of depth was less marked in the first year than in the second. On this soil, maximum yields the first year were obtained from the plots 70 cm. deep. A further increase in depth produced no significant increase in yield. In the second year, on the other hand, as no fertilizer was applied and rainfall was very limited, the crop could obtain more water and nutrients from the deeper soils than from the shallower soils, and depth of solum probably became an indirect requisite for supplying water and nutrients to the crop. The maximum yield was not obtained at a depth of less than 100 cm. In the case of the purple soil, the effect of depth on the growth of wheat during the second year was similar to that in alluvial soil. In the first year, however, the effect was not very pronounced at depths of less than 70 cm. but became marked with a further increase in depth. The maximum yield was not obtained within the range of depths tested.

#### SUMMARY AND CONCLUSIONS

An experiment, in the design of a 3 by 8 split-plot randomized block, was conducted at Pehpei, Szechuan, to study the effect of depth of soil on yield of wheat. Chungking clay loam (a young neutral purple soil), Kialingkiang very fine sandy loam (a young calcareous alluvial soil), and Tsinyunszu sandy clay, loam (a podzolic yellow earth) were used as three main treatments. The sub-treatments were afforded by the following eight levels of soil depth: 10, 15, 20, 30, 40, 50, 70, and 100 cm. From the results obtained during the years 1942-1944, the following conclusions may be drawn:

Of the three soil types tested, the subsurface soil of the podzolic yellow earth was the least fertile. The two young soils were highly productive and were therefore suitable for wheat growth.

The effect of depth of soil on yield of wheat was very pronounced for the two young soils but was less marked for the podzolic yellow earth. The infertility of the podzolic soil might be a limiting factor for the growth of wheat.



# EROSION AS A FUNCTION OF THE SIZE OF THE IRRIGATING STREAM AND THE SLOPE OF THE ERODING SURFACE

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It is apparent to every farmer that serious damage results in attempting to irrigate steep slopes unless the stream is very small. On the other hand, where the slope is gentle, very little erosion takes place even though the stream is comparatively large. When a soil is in good tilth it is susceptible to rapid erosion, but with small streams there is a definite tendency for the eroding surface to stabilize. When, however, the stream exceeds what is here designated as *the critical stream*, the soil continues to wear down indefinitely.

It is of vital importance to find a means of estimating the rate at which the soil will wear down with various sizes of stream and on various slopes, and the depth to which it will wear down during the process of stabilization. The equation of continuity, which expresses the law of conservation of matter, has great practical significance as a guide for experimental procedure. If  $\sigma$  is introduced as the silt content per unit area of the eroding surface and  $T$  as the depth to which the soil has worn down at time  $t$  and at the distance  $x$  down the slope, this equation takes the simple form

$$\frac{\partial T}{\partial t} = \frac{\partial \sigma}{\partial t} + \frac{\partial(v\sigma)}{\partial x} \quad (1)$$

$v$  being the average velocity of the stream at point  $x$  and at time  $t$ . It is apparent that the time at which the water front reaches a point  $x$  will depend upon the choice of  $x$ , and it is found desirable therefore to interpret  $t$  as representing the time measured from the time at which erosion begins.

It is to be noted that the left-hand member of the equation represents the time rate at which silt enters the stream per unit area of the eroding surface. The first term on the right represents the time rate at which silt accumulates in the water lying over a unit area. The last term accounts for the excess of silt being carried beyond this unit area of the stream over that which comes in at the upstream boundary of the water lying over this unit area.

This equation applies in the field as well as in the laboratory, but, for reasons that will become apparent, it is necessary to conduct preliminary experimental tests in the laboratory. If we can succeed in obtaining a successful empirical equation giving the silt content as a function of  $x$  and  $t$ , the size of the stream  $q$ , the slope of the eroding surface  $s$ , together with fundamental soil parameters, it will be possible to eliminate  $\sigma$  from the equation of continuity and thus obtain a partial differential equation with but one dependent variable,  $T$ . This is, in fact, the essential purpose of this study.

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## EXPERIMENTAL PROCEDURE

Serious difficulties are encountered in attempting to measure the silt content of the water from point to point along the stream without disturbing the flow pattern and thus influencing the rate of erosion. As a substitute procedure in the laboratory, flumes of varying lengths are used. The silt content of the water

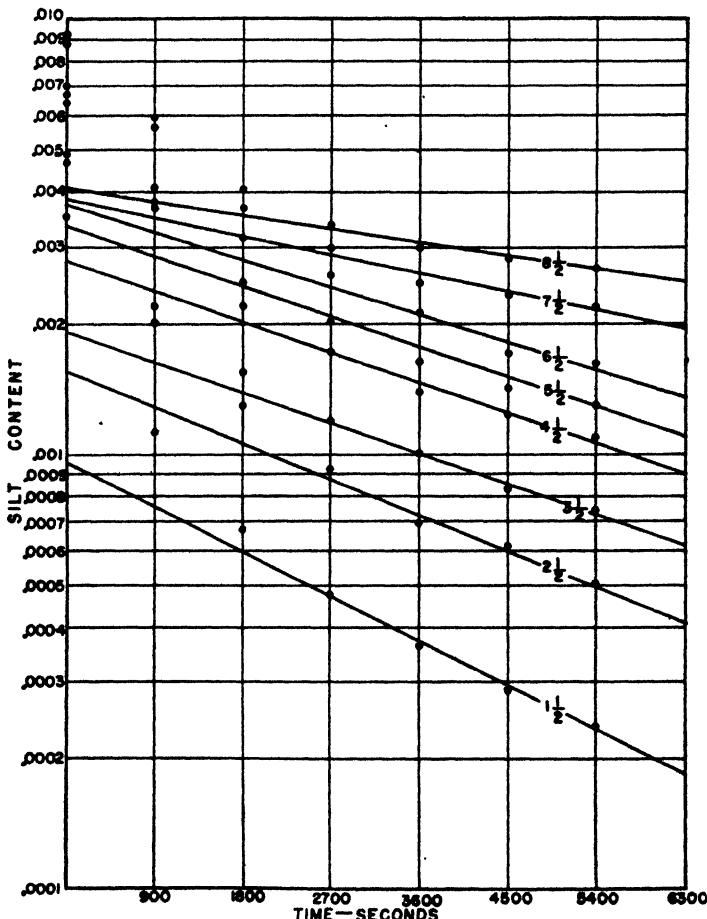


FIG. 1. TYPICAL SET OF SILT-TIME DATA OBTAINED FROM THE RECORDING INSTRUMENT

at the downstream end of the various flumes is measured from time to time with the presumption that, with care in avoiding "end" effects, these measurements will represent with a reasonable degree of precision the actual silt content in the one long flume at distances down the slope corresponding to the flume lengths.

Flumes 20 inches wide were used originally, but more recent data were obtained with flumes 1 foot wide. The soil is air-dried and screened to pass through what corresponds approximately to a number 10 sieve. In this condition the soil is placed on the flume and leveled to a uniform thickness with a blade operated from a fixed guide mounted on the flume. Prior to beginning a test, the

soil is wetted carefully by applying a small stream of water at the upper end and allowing it to creep down the slope under the influence primarily of capillary attraction. The stream is shut off when it reaches the end of the flume, and the soil is allowed to set for approximately three-quarters of an hour before the beginning of a test. It would be expected, of course, that the soil parameters would, in general, depend upon this initial treatment as well as upon the general character of the soil. It should be observed that  $v$  is substantially constant

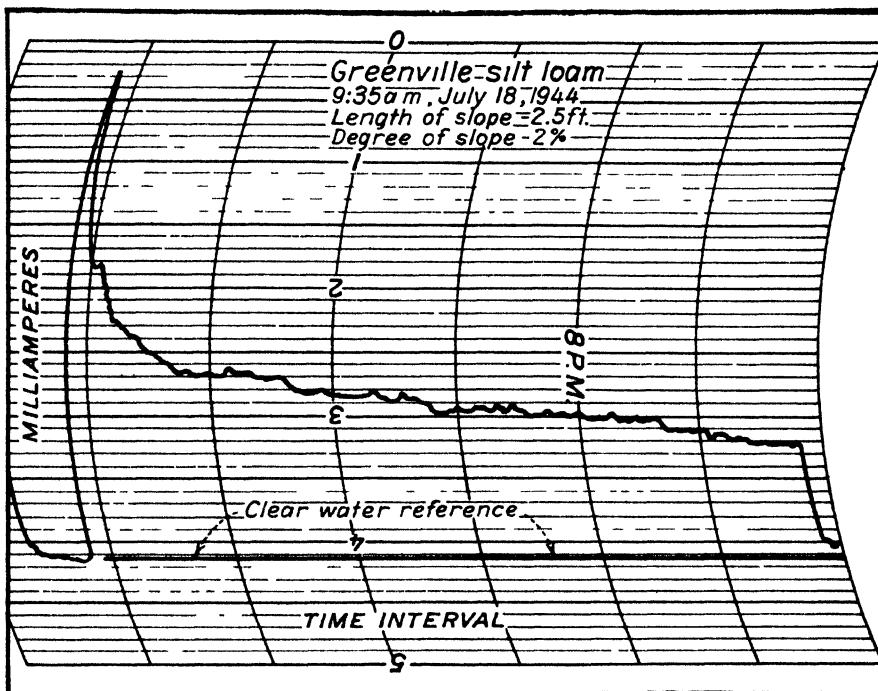


FIG. 2. SAMPLE MEASUREMENT, SHOWING THE INCREASE IN CURRENT WITH TIME, PRODUCED BY LIGHT SOURCE ON A PHOTOELECTRIC CELL AFTER PASSING THROUGH A COLUMN OF THE RUNOFF WATER

The increase in current is proportional to the decrease in soil material in the runoff water with flumes, inasmuch as the size of the stream remains constant except for evaporation loss.

A photoelectric cell is used to measure the silt content. The current from the cell is amplified sufficiently to operate a Westinghouse GX-40 recorder. Samples are collected at intervals, and the actual silt content is determined by weighing and drying, to provide a means of calibrating and checking the current curves.

The diagram of figure 1, plotted on semilogarithmic paper, illustrates a typical set of silt-time data obtained from the recording instrument. It is to be noted that over an initial period of about 20 minutes the rate of decrease of silt with time in most cases is abnormally high, but subsequent points seem to follow straight lines rather closely.

The diagram of figure 2 shows a typical record of the amplified current in

which a comparatively small stream and small slope were used with Greenville silt loam. Numerous curves of this general character were obtained, indicating rather emphatically that with small streams there is an inevitable tendency for the stream to become clear.

#### ANALYTICAL PROCEDURE

Two important features become apparent which suggest an algebraic equation for expressing  $\sigma$  as a function of  $x$  and  $t$ :

1. The silt content of the water at the water front increases as the water front advances down the slope but at a rate that diminishes with  $x$ .
2. For streams that are not excessive the silt content of the water at a random point diminishes with time toward an asymptotic value of zero.

Equation (2) purports to express the first feature,

$$\sigma_0 = \sigma_\infty(1 - e^{-\alpha x}), \quad (2)$$

and equation (3) combines the two features,

$$\sigma = \sigma_0 e^{-f t}, \quad (3)$$

$\sigma_0$  representing the silt content at the water-front,  $\sigma_\infty$  the silt content of the stream when loaded to capacity,  $f$  a function of  $x$  to be determined, and  $t$  the time measured from the time the stream reaches the point  $x$ .

Combining equations (2) and (3) with (1) leads to<sup>2</sup>

$$\frac{\partial T}{\partial t} = \sigma \left( \frac{v \alpha e^{-\alpha x}}{1 - e^{-\alpha x}} - vt df/dx \right). \quad (4)$$

When integrated, on the assumption that  $T = 0$  when  $t = 0$ , this becomes,

$$T = T_\infty(1 - e^{-f t}) + \frac{v \sigma t df/dx}{f} \quad (5)$$

in which  $T_\infty$  is the asymptotic value of  $T$ . It may be expressed thus,

$$T_\infty = v \sigma_\infty \left[ \frac{e^{-\alpha x} (df/dx + \alpha f) - df/dx}{f^2} \right]. \quad (6)$$

If it may be assumed that  $T_\infty$  is independent of  $x$ , that is, that the soil will wear down ultimately the same depth all along the slope, the expression in brackets on the right-hand side of (6) must be constant, and this leads therefore to the differential equation,

$$\frac{e^{-\alpha x} (df/dx + \alpha f) - df/dx}{f^2} = K, \quad (7)$$

and this has for a solution,

$$f = (\beta/x)(1 - e^{-\alpha x}). \quad (8)$$

<sup>2</sup> Inasmuch as  $t$  is measured from the time the water front reaches the point  $x$ , this time  $t$  is related to the actual time  $t'$  by the relation,  $(t' - t) = x/v$ , and from this we obtain by differentiation,  $\partial t/\partial x = -1/v$ ,  $t'$  being independent of  $x$ .

It is apparent that  $\sigma_\infty$ ,  $\beta$ , and  $\alpha$  may change with  $q$  and  $s$  and with the character of the soil. It may be seen also from equation (3) that when  $f$  is zero  $\sigma$  is equal to  $\sigma_0$  for all values of  $t$ , and this will be true when the stream is at or beyond the critical value. From equation (8) it may be seen that when  $\beta$  vanishes  $f$  must also vanish, and with the aid of equations (4) and (8) it may be seen that for values of  $q$  beyond the critical value equation (4) takes the special form,

$$\frac{\partial T}{\partial t} = v\sigma_\infty \alpha e^{-\infty}. \quad (9)$$

At the clear-water intake where  $x = 0$ , this reduces further to

$$\frac{\partial T}{\partial t} = v\sigma_\infty \alpha. \quad (10)$$

Also, if we combine equation (8) with equation (6), we obtain,

$$T_\infty = v\sigma_\infty / \beta. \quad (11)$$

Equations (2) and (3) must, of course, be construed as having tentative significance, although they seem to conform reasonably well with a very considerable number of experimental data for soils of varying character. If, therefore, we can succeed in obtaining the functions,

$$\sigma_\infty = \sigma_\infty(q, s, \lambda, \mu, v, \dots) \quad (12)$$

$$\alpha = \alpha(q, s, \lambda, \mu, v, \dots) \quad (13)$$

$$\beta = \beta(q, s, \lambda, \mu, v, \dots), \quad (14)$$

$\lambda$ ,  $\mu$ ,  $v$ , etc., representing the fundamental soil parameters, and of measuring these parameters for various soil types, we shall have succeeded in making a first substantial step toward the goal sought.

It is to be noted that the silt content of the water per unit volume is more readily measured than the silt content per unit area of the eroding surface; also that the size of the stream  $q$  per unit width of flume is more readily measured than the velocity  $v$ . It is convenient therefore to rewrite the practical formulas (10) and (11) in the forms,

$$\frac{\partial T}{\partial t} = q\sigma_\infty' \alpha \quad (15)$$

$$T_\infty = q\sigma_\infty' / \beta, \quad (16)$$

the symbol  $\sigma_\infty'$  now representing the silt content per unit volume rather than per unit area of the eroding surface.

In order to illustrate the practical significance of equations (15) and (16), the following special tentative forms were assumed for the functions of equations (12), (13), and (14).

$$\sigma_\infty' = a s^m q^n \quad (17)$$

$$\alpha = \alpha \quad (18)$$

$$\beta = \beta_0(1-q/q_c) \quad (19)$$

$\alpha$  being included (tentatively) with  $a$  and  $\beta_0$  as soil parameters. The symbol  $q_c$

represents the critical value of  $q$ , and is taken to be inversely proportional to the  $\gamma$ 'th power of  $s$ , thus,

$$q_c = b/s^\gamma, \quad (20)$$

the factor  $b$  being a soil parameter.

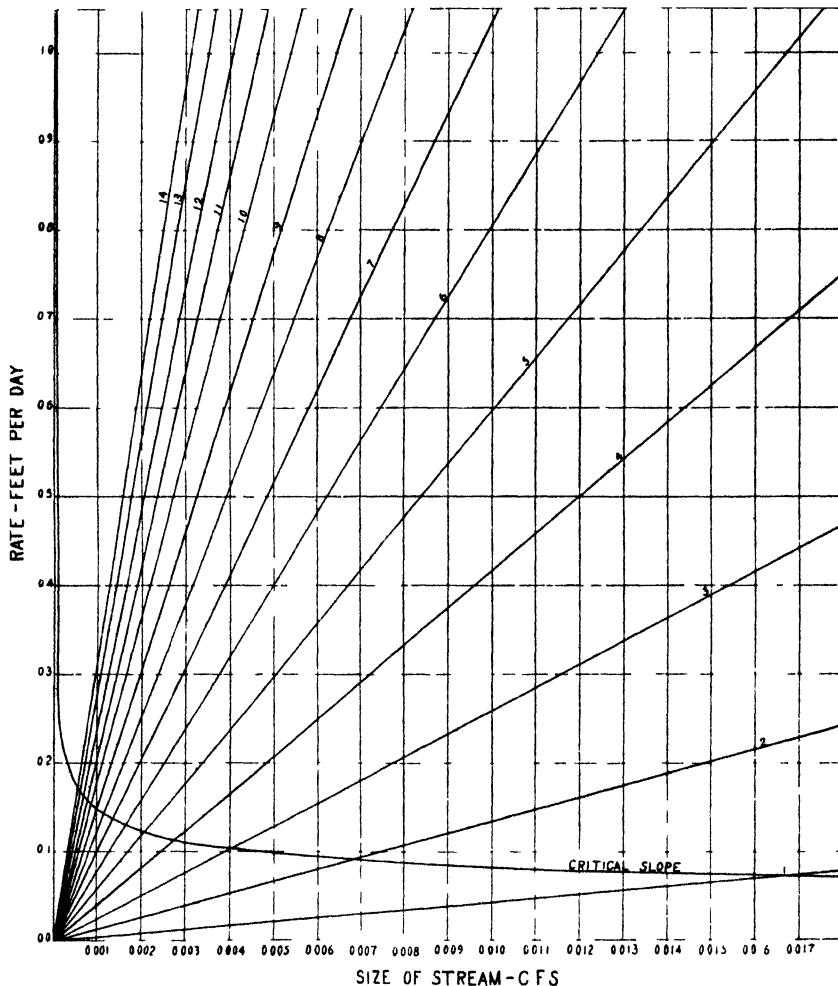


FIG. 3 RATE AT WHICH THE SOIL WEARS DOWN, THE SLOPE BEING THE INDEPENDENT VARIABLE AND THE SIZE OF THE STREAM THE PARAMETER

The points along the curve marked "critical stream" correspond to the erosion rates for streams that are critical for the various slopes

If  $\sigma_w'$  has the dimensions of volume of silt per unit volume of water, the parameter  $a$  is dimensionless provided  $n$  is taken to be equal to zero;  $\alpha$  has the dimensions of the reciprocal of length,  $\beta_0$  the dimensions of velocity, and  $b$  the dimensions of  $q$ . Making use of the foot for the unit of length and the second for the

unit of time, the following tentative values<sup>3</sup> were obtained for the soil parameters for the Greenville soil:

$$a = 1.76$$

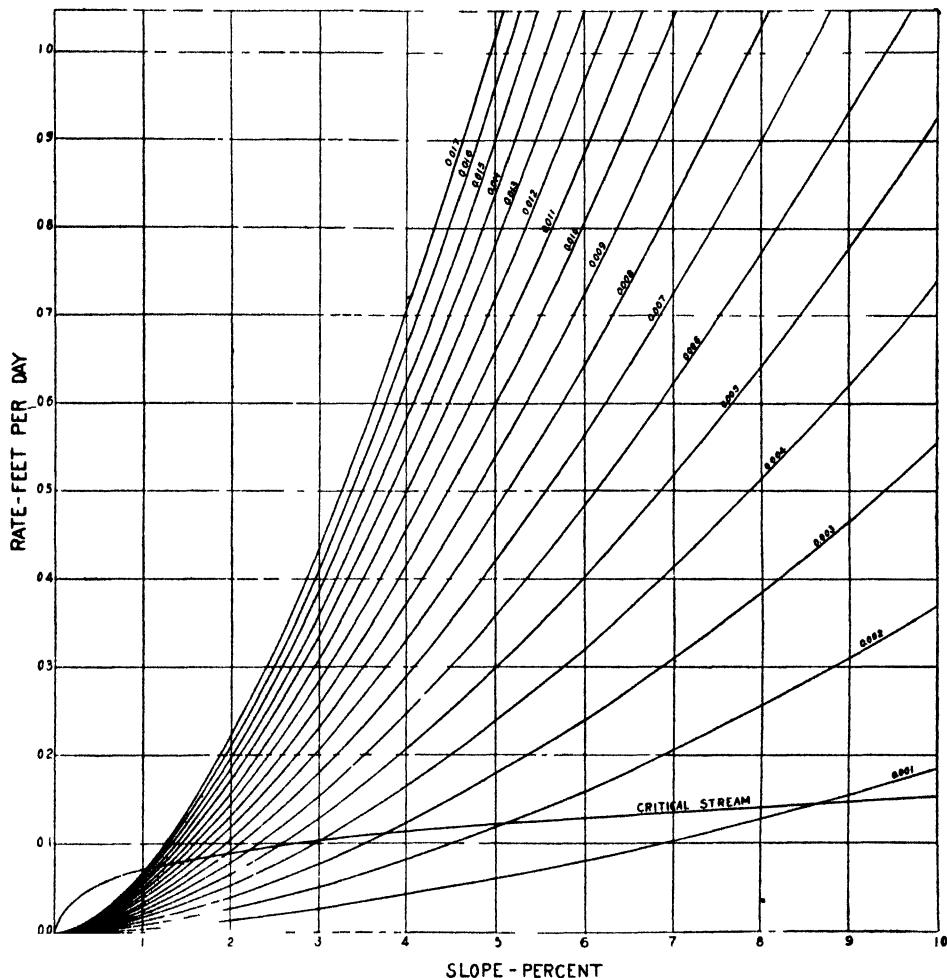


FIG. 4 SAME AS FIGURE 3, EXCEPT THAT THE SIZE OF STREAM IS THE INDEPENDENT VARIABLE, THE SLOPE BEING THE PARAMETER

The points along the curve marked "critical slope" correspond to erosion rates for slopes that are critical for the various sizes of stream

$$b = 4.22 \times 10^{-5}$$

$$\alpha = 0.052$$

$$\beta_0 = 2.77 \times 10^{-3}$$

<sup>3</sup> Utah Agricultural Experiment Station Bulletin 320 by O. W. Israelsen *et al.* presents the results of some field studies which seem to indicate that the analytical attack may be extended to the problem of irrigation design.

and the following values for the constants,  $\gamma$ ,  $m$ , and  $n$ ,

$$\begin{aligned}\gamma &= 1.3 \\ m &= 1.63 \\ n &= 0.\end{aligned}$$

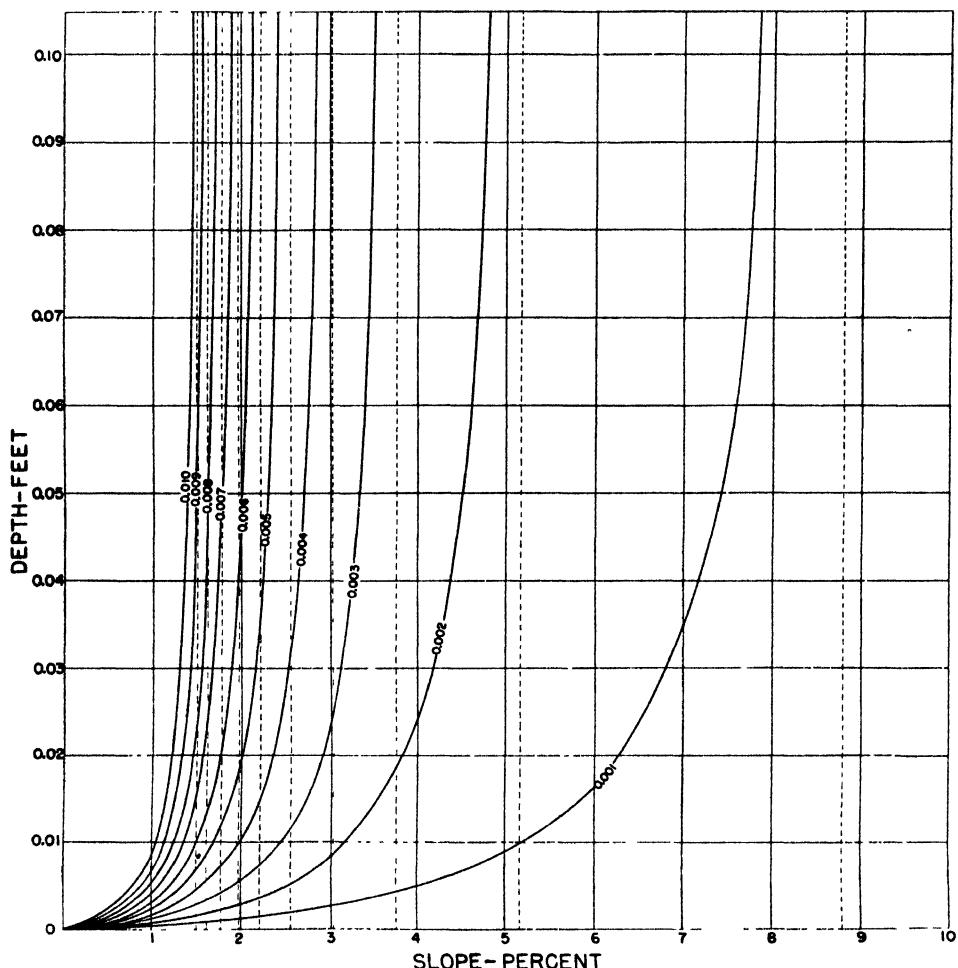


FIG. 5. DEPTH TO WHICH THE SOIL WEARS DOWN DURING THE PROCESS OF STABILIZATION.  
THE SLOPE IS THE INDEPENDENT VARIABLE, THE SIZE OF THE STREAM  
BEING THE PARAMETER

If we replace the functions  $\sigma_\infty'$ ,  $\alpha$ , and  $\beta$  in equations (15) and (16) by the right-hand members of equations (17), (18), and (19), and multiply the right-hand members of equation (15) by a factor of 86,400 to obtain the rate of erosion, per day, rather than per second, and finally substitute the numerical values given, these equations take the simple form:

$$\frac{\partial T}{\partial t} = 7900 s^{1.63} q \quad (21)$$

$$T_{\infty} = \frac{636 \text{ sq}}{1 - 23,700qs^{1.8}}. \quad (22)$$

If, finally, we impose the condition that the  $q$  of equation (21) shall be critical,

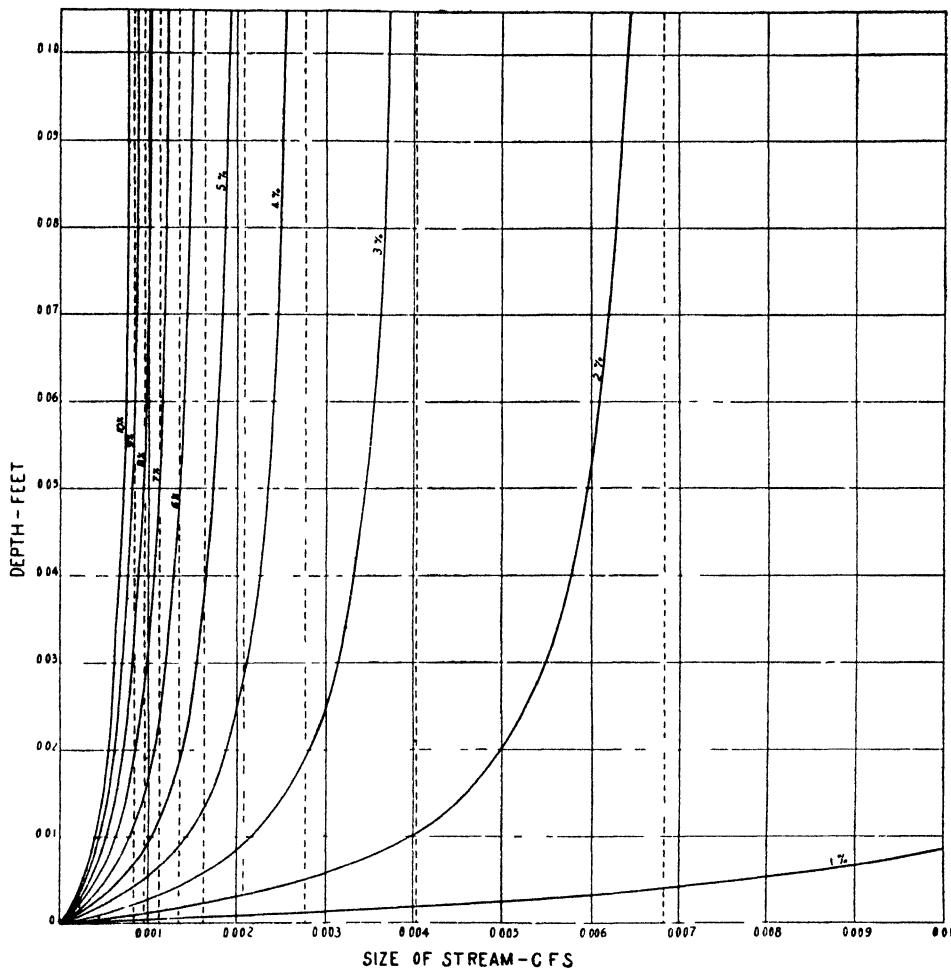


FIG. 6. DEPTH TO WHICH THE SOIL WEARS DOWN DURING THE PROCESS OF STABILIZATION.  
THE SIZE OF THE STREAM IS THE INDEPENDENT VARIABLE, THE SLOPE  
BEING THE PARAMETER

and eliminate, in turn,  $q$  and  $s$  by means of equation (20), we obtain the two critical equations,

$$(\partial T / \partial t)_c = 0.333s^{0.83} \quad (23)$$

$$(\partial T / \partial t)_c = \frac{0.0261}{q^{0.253}}, \quad (24)$$

giving the rate at which the soil wears down at the clear-water intake when the stream is at the critical value.

In order to obtain the erosion rate at any point along the flume when  $q$  is critical, it would be necessary only to include the factor  $e^{-\alpha s}$  of equation (9). In the field, however, the size of the stream decreases with distance down the slope at a rate that depends upon the permeability of the soil, and at some point along the slope the stream would become saturated and beyond this point silt would deposit. It seems logical to presume that, as time goes on, this critical point would move down the slope, approaching the point where  $q$  vanishes asymptotically.

In figures 3 and 4, equation (21) is plotted; in the one case  $q$  is the independent variable with  $s$  a parameter, and in the other,  $s$  is the independent variable and  $q$  is the parameter. The curve of equation (23) is superposed in the first diagram and the curve of equation (24) in the other.

The diagrams of figures 5 and 6 represent  $T_\infty$  in the same way, except that the critical values of  $s$  and  $q$  are indicated by vertical broken lines. It is apparent, for example, that as the slope for a given stream approaches the value that is critical for the stream, the ultimate or asymptotic depth to which the soil wears down tends toward infinity; or that as the size of the stream for a given slope approaches the value that is critical for the slope, the ultimate depth to which the soil will wear down becomes infinite.

#### CONCLUSIONS

It should be apparent that there does exist an analytical approach toward the solution of the erosion problem that will prove to be valuable to farmers in the design of their irrigation systems. A very considerable number of experimental data for Greenville silt loam have been obtained in the laboratory which seem, qualitatively at least, to conform with equation (3), and, although the assumptions of equations (17), (18), (19), and (20) are subject to such modifications as may prove to be necessary, there is good reason to believe that some such approach as this will be found suitable and satisfactory.

# INFLUENCE OF EVACUATION UPON LABORATORY PERCOLATION RATES AND WETTING OF UNDISTURBED SOIL SAMPLES<sup>1</sup>

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In measuring the laboratory percolation rates of undisturbed soil cores or lumps, the primary objective is to obtain results that will indicate real differences among samples or conditions as they occur in the field. It would be highly desirable if the laboratory measurements could be converted directly into field percolation or permeability values, but this can be done only in a very general way. In the field, such factors as the arrangement and depth of the various soil horizons and the action of trapped air are likely to introduce unexpected variations in soil profile permeability which cannot be satisfactorily evaluated in the laboratory. Even though the limitations of laboratory values are recognized and the results considered to be only relative, there are serious complications in the determinations. One of the greatest of these is trapped air, which has been shown to occupy considerable pore space after ordinary laboratory wetting procedures. The question naturally arises as to whether this air should be removed, and if removed, what influence removal has upon percolation measurements.

In a study of laboratory-packed samples, Christiansen (1) showed that when air had been evacuated, the samples had highest percolation rates at the beginning of the measurement. For nonevacuated samples, the highest values were obtained several days after percolation had started, at a time which seemed to coincide with the removal of virtually all the entrapped air. He showed further that the percentage increase of percolation corresponded closely with the actual amount of air removed from the various soils. He pointed out that with some soils the final minimum percolation rate was considerably lower for the vacuum-wetted samples than for those wetted in the open air.

The results reported in this paper show some of the influences of trapped air in working with undisturbed samples. Both advantages and disadvantages associated with removal of such air are indicated in an attempt to help clarify the usefulness of different percolation measurements.

<sup>1</sup> Contribution of Soil Conservation Service Research, U. S. Department of Agriculture, and of the agronomy and genetics department, West Virginia Agricultural Experiment Station. Published with the approval of the director of the Agricultural Experiment Station as Scientific Paper No. 346.

<sup>2</sup> Project supervisor, Soil Conservation Service; and assistant soil technologist, Soil Conservation Service, and assistant in agronomy, West Virginia Agricultural Experiment Station, respectively.

#### SAMPLES AND METHODS

All results were obtained with soil in its natural physical state. Samples were collected in the field either as 3-inch cores or as natural lumps and were preserved in moisture-proof containers. A few drops of toluene were used to prevent fungus growth until the determinations were made. Sampling and general procedures are essentially the same as those already described (5). Evacuation consisted of placing field-moist samples in a vacuum chamber and applying a standard water jet pump to obtain 0.7 to 0.8 atmosphere of suction. After a few minutes, distilled water was slowly admitted through a T-junction in the suction line and the samples were left standing in the water until they appeared to be completely wet. Usually they remained in the chamber overnight, but the time varied with the type of samples. Massive clays require more than one day for complete wetting.

All percolation rates are expressed in inches per hour, and "unit head" indicates that the ratio of the hydraulic head to the length of the sample is 1. This permits a simple conversion of the percolation rates into any type of permeability factor such as the Darcy coefficient of permeability.

#### RESULTS

##### *Influence of the hydraulic head*

Ever since percolation rates have been run in this laboratory, it has been noted that samples wetted in the open air fail to show a close relationship between the hydraulic head and the rate of percolation as required by Darcy's law. Several comparisons are summarized in table 1. In many cases the increased head causes a greater increase than the law requires, and in some cases, less increase. This is disturbing because it seems to indicate that one standard head must be used in all cases regardless of the rate, and it leaves a feeling of uncertainty as to the meaning of the values obtained, for if a measured value is so strongly influenced by some uncontrolled factor that a fundamental hydraulic law fails to apply, there seems to be little assurance that the value at one particular head has much application even as a relative index.

In the study of evacuated samples, it has been evident that the percolation rate is less erratic relative to the hydraulic head. Several comparisons appear in table 2, which, though showing some deviations, indicate a fairly satisfactory linear relation between the rate and the head for widely different types of samples. Some of the deviations are undoubtedly experimental errors involving some fluctuation of the head and inaccuracies in reading small volumes. But even including the errors, the rates at different heads are sufficiently close to theoretical values to serve most practical purposes, especially when the normal differences among replicate samples are considered. It is also possible that some deviations may be due to an incomplete removal of the trapped air, since in 25 typical comparisons the total water content after evacuation lacked 3.5 per cent of equalling the calcuated pore spaces.

The relation of the percolation rate to the hydraulic head for evacuated samples is perhaps more clearly evident in figures 1, 2, 3, and 4. Variable samples and rates are represented. The errors of determination are greater for the slow rates and short time intervals.

TABLE 1

*Laboratory percolation rates determined at successive short-time intervals at different hydraulic heads, for a variety of undisturbed soil lumps or cores wetted in the open air, showing typical irregular divergences from Darcy's law*

Percolation in inches per hour

SAMPLE DESCRIPTION	PERCOLATION RATES		
	1 hydraulic head	4 hydraulic heads	10 hydraulic heads
Compact surface	0 6	2 0	
Porous surface	40 0	120 0	
-			
Silt to clay subsoils	0 37 0 75 0 15 0 50 0 20 0 015	6 5 1 9 0 5 1 6 1 2 0 05	
Clay subsoil	0 008 0 008	0 35 0 17	
Silty subsurface	0 50	3 5	
Compact subsoil	0 01	0 05	
Aggregated subsoil	0 9 0 5 5 5 3 5	4 0 2 0 11 0 12 0	
Surface silt loams	0 4 11 0 23 0	1 0 22 0 77 0	
Compact subsoil		1 7 0 5	5 5 1 7
Slack water clay		0 008	0 12
Silty subsoil		2 0 1 0	6 5 2 7
Aggregated subsoil	0 36	1 8	
Compact subsoil	0 006	0 02	

*Influence of time upon percolation*

It is recognized that long-continued percolation through samples wetted in the open air gives rather large changes in rates. A number of trials with evacuated samples in their natural physical state seem to indicate that changes are relatively small for a considerable length of time.

Figures 1, 2, 3, and 4 show some fluctuations, but the initial rates are near

maximum in all cases, as indicated by Christiansen for laboratory-packed samples (1).

Both a silty and a well-aggregated clay subsoil show a definite decline in percolation (fig. 5), although the initial rate is relatively stable for a considerable

TABLE 2

*Laboratory percolation rates through undisturbed soil samples at different hydraulic heads, after wetting under a vacuum*

Percolation in inches per hour

SOIL MATERIAL	PERCOLATION RATES			
	1 hydraulic head	2 hydraulic heads	4 hydraulic heads	8 hydraulic heads
Massive red clay.....			0.0023	0.0043
Aggregated clay.....	0.9	2.4		
Surface loam.....	2.4	6.0		
Massive gray clay.....		0.001†	0.002	
Massive gray clay.....		0.003	0.008	
Aggregated subsoil.....	1.20	2.45	4.80	
	0.55	1.2		
	0.16	0.40	0.87	
Silty subsoil.....	0.20	0.44		
		0.04	0.10	

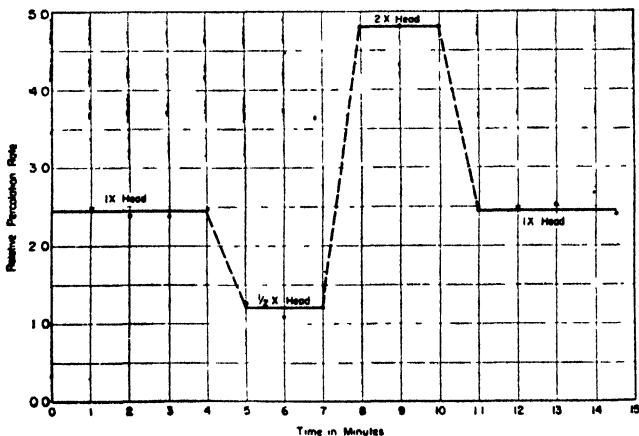


FIG. 1. LABORATORY PERCOLATION THROUGH A WELL-AGGREGATED CLAY SUBSOIL LUMP AT DIFFERENT HYDRAULIC HEADS, AFTER WETTING UNDER A VACUUM  
Readings at successive time intervals

time. The final rate for the aggregated clay (Hagerstown subsoil) is very slow, being essentially the same as for massive clay. This seems to indicate that the water passages among structural units, which are normally distinct in this soil, have completely lost their effectiveness after long-continued percolation fol-

lowing the removal of air. This has interesting implications relative to the development and maintenance of the well-aggregated structural condition.

The final rate of the silty subsoil is considerably higher than that of the aggregated clay, although the initial rate was lower. Apparently the final rate rep-

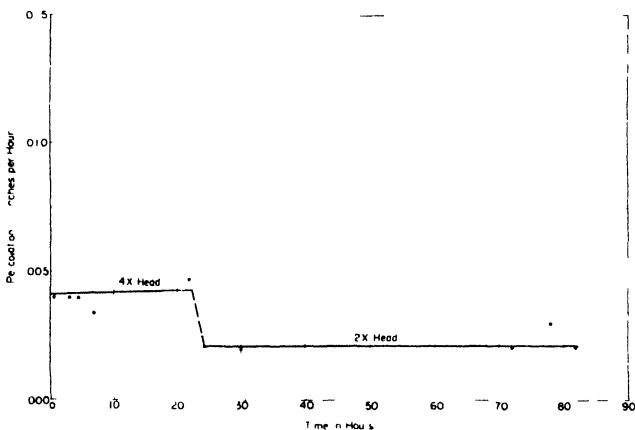


FIG. 2 LABORATORY PERCOLATION THROUGH A MASSIVE GRAY CLAY SUBSOIL LUMP AT TWO DIFFERENT HYDRAULIC HEADS AFTER WETTING UNDER A VACUUM  
Readings at irregular intervals as shown

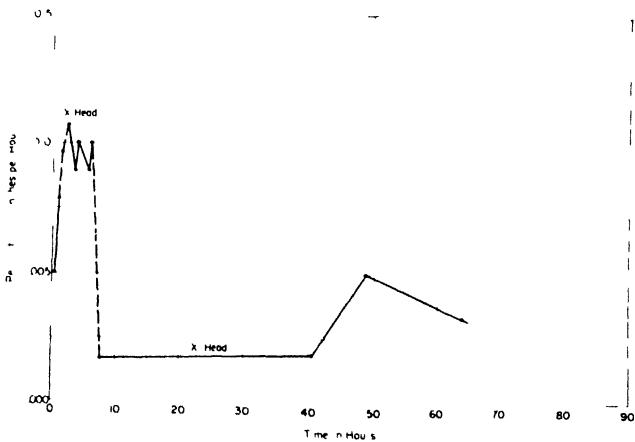


FIG. 3 LABORATORY PERCOLATION THROUGH A MASSIVE ACID RED CLAY TERRACE SUBSOIL AT TWO DIFFERENT HYDRAULIC HEADS  
Readings at successive irregular intervals

resents the movement through the closely packed silty textural units whereas the initial rate reflected some structural arrangement which permitted greater movement. This subsoil is a moderately developed "silt pan" and in the field is considered rather slowly permeable, certainly less permeable than the normal Hagerstown subsoil. The relative initial rates of this subsoil, the Hagerstown

subsoil, and the massive clay (fig. 5) are much more consistent with relative field permeabilities than the final rates which would place Hagerstown as less permeable than the silt pan soil and little different from the massive clay from a soil with very poor internal drainage.

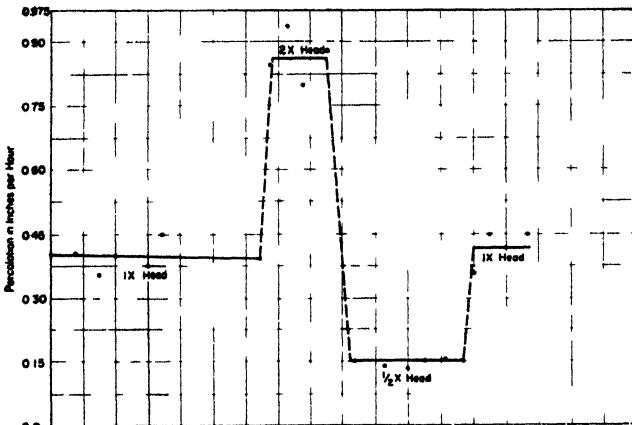


FIG. 4. LABORATORY PERCOLATION THROUGH A NATURAL LUMP OF SILTY SUBSOIL AT DIFFERENT HYDRAULIC HEADS, AFTER WETTING THE SAMPLE UNDER A VACUUM

Readings at successive time intervals

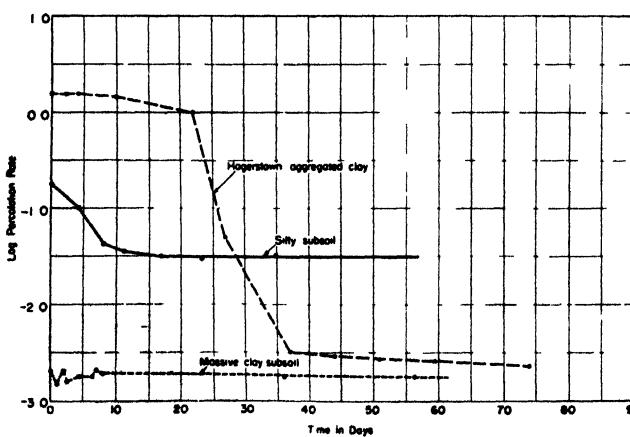


FIG. 5 INFLUENCE OF TIME ON THE PERCOLATION RATE OF THREE TYPES OF SUBSOILS, FOLLOWING EVACUATION AND WETTING

*Influence of evacuation upon absolute percolation rates and replications*

Table 3 shows some comparative rates of percolation for several different soil materials. The first surface samples listed were all sod cores which showed little evidence of physical change during wetting and rewetting. It seems clear that the removal of air increased percolation through these samples and that

TABLE 3

*Laboratory percolation rates of natural soil cores or lumps from several different soil horizons wetted in the open air, compared to the same or similar samples wetted under a vacuum*

Percolation rates in inches per hour at unit head

*Litz silt loam surface soil from sod*

OPEN-AIR WET	VACUUM WET*	DIFFERENCE
1.9	2.4	+0.5
0.7	7.0	+6.3
0.7	6.1	+5.4
4.0	6.0	+2.0
2.1	5.9	+3.8
5.6	6.6	+1.0
Average.....2.5	Av. ....5.7	
Standard Deviation 1.94	S. D. ....1.65	

*Hagerstown*

OPEN-AIR WET		VACUUM WET	
Subsurface	Subsoil	Subsurface	Subsoil
0.25	0.13	2.5	1.8
7.7	0.50	5.0	0.9
8.8	2.5	1.2	0.05
6.0	1.0	3.0	0.6
6.0	2.5	4.0	1.2
7.0	0.01	2.5	—
0.4	0.12	—	Av. ...0.9
2.4	1.1	Av... 3.0	S.D. .. 0.59
—	3.3	S.D. .. 1.28	
Average..4.8	1.2		
S.D. ....3.34	1.0		
	1.0		
	1.2		
	—		
Av. . 1.2			
S.D. . 0.97			

*Subsoil siltpans*

OPEN-AIR WET	VACUUM-WET
0.03	0.16
0.11	0.09
0.13	0.09
0.25	0.40
0.64	0.06
0.03	0.06
0.05	0.20
0.07	—
—	Av. ... ..... 0.15
Average . . . 0.16	S. D.... ..... 0.12
S. D..... . . 0.20	

\* These surface samples were evacuated and rewet after first being wetted and determined in the open air. All subsurface and subsoil samples are separate samples wetted as indicated.

some apparent differences among the nonevacuated values were due to the influence of the trapped air. The increased percolation due to evacuation is so great in several cases that it can only be accounted for by assuming that air was blocking some of the coarsest pores. This air-trapping in the coarsest pores was indicated in previous work, although the tendency with a wide variety of samples was believed to be toward a concentration of trapped air in the intermediate pore sizes (4). Results by Pillsbury and Appleman (3) seem to indicate a tendency for air-trapping in the intermediate pore sizes in the case of artificially packed samples. Since this packing eliminates large pockets and root or other



FIG. 6. AIR-DRY LUMPS FROM A SILTY SUBSOIL

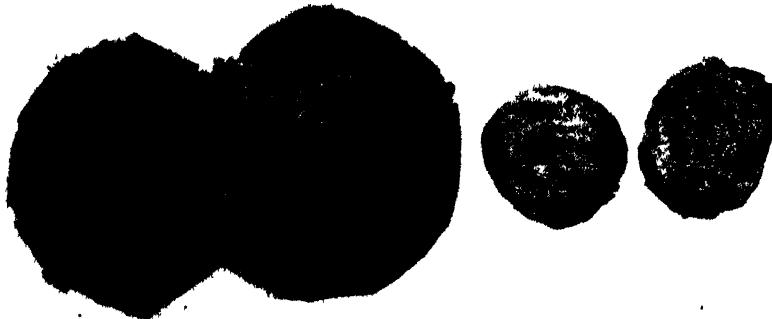


FIG. 7. THE SAME SUBSOIL LUMPS SHOWN IN FIGURE 6, AFTER COMPLETE WETTING

The two lumps on the left were simply immersed in water; the two on the right were evacuated before being brought into the water. The explosive action of trapped air is evidently responsible for the complete physical disruption of the two lumps on the left.

channels, the conclusion seems reasonable that air-trapping is greatest in the intermediate range when the only pores considered are those among natural textural or structural units.

The other comparisons in table 3 are for different samples of the same or closely similar material. Most of these values were obtained with soil lumps. In the three subsoil comparisons the replication seems to be better in all cases with the evacuated samples. The average absolute values are little different, but extremely high and low values are obtained for nonevacuated samples. The low values are believed to be related to air-locked conditions; the high values, to physical disruption and the opening of channels or cracks that would not function

under natural conditions. Often these cracks or disruptions can be readily observed.

#### *Physical disruption due to open-air wetting*

The explosive action of trapped air, which disrupts samples in the Yoder method of aggregate analysis (6) as well as in other dispersion measurements, tends to occur when cores or soil lumps are wetted in the open air. Figures 6 and 7 illustrate the extreme differences between open-air and vacuum wetting which is sometimes obtained with unconfined lumps. Naturally this extreme cannot occur with cores or with confined lumps, but there is a distinct tendency for disruption in open-air wetting. This mechanism is very noticeable with silty subsoils and with well-aggregated clay soils. It has least effect upon surface cores from sod. It is probably somewhat worse for lumps than for complete cores.

#### DISCUSSION OF RESULTS

Any decision regarding the most appropriate laboratory technique for determining the percolation rates of undisturbed soil cores or lumps will undoubtedly be governed by the particular objective of the measurements. If the object is to obtain a relative value as an index of the natural physical conditions of undisturbed soil samples, removal of air by evacuation has decided advantages. This seems to eliminate considerable variation among samples by preventing the physical disruption which is a serious complication in open-air wetting. Physical disruption may be sufficient to alter completely the apparent percolation rates of certain types of samples, particularly subsoils.

Evacuation also seems to give reproducible values which are essentially proportional to the hydraulic head. This permits the use of any convenient head for arriving at a permeability value that can be defined independently of the head, as has been done in permeability studies of geologic materials (2).

Long-continued percolation measurements through evacuated samples with natural structure give little variation with time in the case of massive samples, but a slowly decreasing rate for aggregated samples, which apparently corresponds with a deterioration of the natural structure. This seems to indicate that the best index of the natural structural condition is obtained by the initial rate. The rate and total amount of decline of percolation would be of interest in many types of studies (fig. 5), but in obtaining a relative index of the permeability of samples with natural structure the initial rate is more indicative.

One of the greatest disadvantages of evacuation in making permeability measurements is that the factor of trapped air is largely eliminated, and, insofar as the amount and the influence of the air may be associated with soil differences, this difference is lost. It has already been indicated that the amount of air trapping is associated with various soil and plant factors (4), and there will undoubtedly be important advantages to consideration of this variable in many types of studies.

Since open-air wetting in the laboratory tends to disrupt natural samples much more than evacuation and wetting, the relative results by evacuation are ordinarily more reliable. Continued experience seems to indicate that the utmost care and attention must be given during the collection of samples and in laboratory handling to avoid all compaction, disruption, and puddling when percolation or moisture tension measurements are to be made. The very wide range in normal soil percolation rates offers an opportunity for determining real differences among many soil materials, but an apparently small change due to handling, or an undetected leak may completely upset the accuracy of the permeability measurements. But when a simple permeability test is made in the apparatus described, it is easy to observe whether the rate is due to normal pores or to cracks or channels. In such a measurement, these are the points of interest, and the results seem very helpful in understanding the soil-water relations.

#### SUMMARY

Percolation results are presented for undisturbed soil cores or soil lumps which indicate that evacuation of samples prior to laboratory wetting gives practical agreement between the percolation rate and the hydraulic head as required by Darcy's law in contrast to the poor agreement obtained with samples wetted in the open air.

For evacuated samples, the initial percolation rate is shown to be essentially the maximum rate for a number of samples. Massive clays showed little or no trend with time up to 190 hours, but a silty and an aggregated clay subsoil show a definite decline until a minimum is reached after 8 to 40 days. The declining rate is apparently associated with a deterioration of the natural structure until an essentially massive condition is reached in the case of the aggregated clay (Hagerstown subsoil) and a single-grained condition in the case of the silty subsoil.

For surface sod samples, a definite increase in percolation is shown following removal of air, the increase being quite different for different individual cores, amounting to 10 times the initial rate in two cases. Subsoil comparisons of evacuated and nonevacuated samples show better replication by evacuation, with average values about the same. The relatively poor replication for subsoils wetted in the open air is considered to be caused in part by variable physical disruption and by air-locking. An illustration is given showing that serious disruption is largely avoided by evacuation.

Advantages of wetting by evacuation seem to justify this technique for many purposes, although differences in air-trapping associated with various soils and conditions require much additional study.

#### REFERENCES

- (1) CHRISTIANSEN, J. E. 1944 Effect of entrapped air upon the permeability of soils. *Soil Sci.* 58: 355-365.
- (2) MUSKAT, M. 1937 *The Flow of Homogeneous Fluids Through Porous Media.* McGraw-Hill, New York.

- (3) PILLSBURY, A. F., AND APPLEMAN, D. 1945 Factors in permeability changes of soils and inert granular material. *Soil Sci.* 59: 115-123.
- (4) SMITH, R. M., AND BROWNING, D. R. 1942 Persistent water—unsaturation of natural soil in relation to various soil and plant factors. *Soil Sci. Soc. Amer. Proc.* 7: 114-119.
- (5) SMITH, R. M., BROWNING, D. R., AND POHLMAN, G. G. 1944 Laboratory percolation through undisturbed soil samples in relation to pore-size distribution. *Soil Sci.* 57: 197-218.
- (6) YODER, R. E. 1936 A direct method of aggregate analysis and a study of the physical nature of erosion losses. *Jour. Amer. Soc. Agron.* 28: 337-351.



# EFFECT OF LIMING DIFFERENT SOIL LAYERS ON YIELD OF ALFALFA AND ON ROOT DEVELOPMENT AND NODULATION<sup>1</sup>

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Despite the fact that soil liming is very generally practiced in the humid sections of the United States, most investigations on soil acidity and liming have been concerned only with the acidity of the surface soil. Since many of the commonly grown crops have root systems that extend well into the subsoil, it is not surprising that in certain instances there has been a lack of correlation between the acidity of the surface soil and crop response to liming. It has been noted that reasonably good stands of alfalfa and sweet clover often occur on some of the limestone soils in West Virginia even though the surface soil is strongly acid. These observations led to an attempt to measure the effect of additions of lime at various depths upon crop growth. Because of the fact that alfalfa responds well to liming on acid soils and because it has a deep root system, this crop was selected for the study.

## PLAN OF EXPERIMENT

The experiment was conducted in large glazed sewer tiles, 24 inches in diameter and 27 inches deep. These were placed in the ground, bell downward, to a depth of about 25 inches. A 3-inch tile line was placed below each sewer tile and covered with 2 inches of gravel to facilitate drainage.

The soil selected for use was strongly acid Gilpin silt loam in a relatively low state of fertility. In collecting the soil, the top 8-inch layer was first removed, next the 8-16-inch layer, and finally the 16-24-inch layer. Each layer of soil was thoroughly mixed, and samples were taken to the laboratory for certain chemical analyses. The analyses are given in table 1.

The amount of calcium hydroxide required to produce the desired pH values of the various layers was calculated from the buffer curve. The detailed plan of treatments is given in table 2.

The lime was added about the first of March and the soil kept moist in piles until the middle of May when it was placed in the tiles. In filling the tiles the equivalent of 150 pounds of dry soil from the 16-24-inch layer was first added and packed gently. The same procedure was followed successively for the

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8-16-inch and the 0-8-inch layers. Previous calculations had shown that this amount of soil would make approximately 8-inch layers in the tiles.

After the tiles were filled, superphosphate (20 per cent  $P_2O_5$ ) and muriate of potash were applied at the rates of 600 and 100 pounds per acre respectively.

TABLE 1  
*Chemical analyses of Gilpin silt loam*

DEPTH Inches	TOTAL EXCHANGE CAPACITY*	EXCHANGEABLE BASES*				BASE SATURATION per cent	pH	Ca/Mg
		Ca m.e.	Mg m.e.	K m.e.	Total m.e.			
0-8	7.98	2.11	0.16	0.38	2.81	35.2	4.86	13.2
8-16	7.57	3.49	0.61	0.35	4.52	59.7	5.06	5.7
16-24	7.16	4.23	1.02	0.28	5.57	77.8	5.03	4.1

\* On basis of 100 gm. of soil.

TABLE 2  
*Detailed plan of treatments*

TREATMENT NUMBER	APPROXIMATE pH VALUES DESIRED*			FERTILIZER ADDED†
	0-8-inch layer	8-16-inch layer	16-24-inch layer	
1	5.0	5.0	5.0	PK
2	5.0	7.0	5.0	PK
3	6.0	5.0	5.0	PK
4	6.0	6.0	5.0	PK
5	6.0	7.0	5.0	PK
6‡	6.0	5.0	5.0	PK
7	6.0	5.0	7.0	PK
8	6.0	6.0	7.0	PK
9	7.0	5.0	5.0	PK
10	7.0	6.0	5.0	PK
11	7.0	7.0	5.0	PK
12‡	7.0	5.0	5.0	PK
13	6.0	6.0	5.0	P
14	6.0	6.0	5.0	None

\* To obtain pH values of approximately 6.0 in the 0-8- and 8-16-inch layers, 3,300 and 1,800 pounds  $Ca(OH)_2$  per acre (2,000,000 pounds) respectively were required; to obtain pH values of 7.0 in the 0-8-, 8-16-, and 16-24-inch layers, 10,000, 4,500, and 7,400 pounds  $Ca(OH)_2$  per acre, respectively, were needed.

† P = 600 pounds per acre 20 per cent superphosphate, K = 100 pounds KCl per acre.

‡ Surface 3 inches only limed to values indicated.

These were worked into the surface 3 inches of soil. All treatments were in triplicate. Alfalfa was planted in the spring of 1930. A good stand was obtained, and one cutting was made in the fall. Three cuttings annually were removed from 1931 to 1933, inclusive. The yields in 1933 were highly variable because of a thinning stand. The tiles were reseeded in 1934 but, since only a

partial stand was obtained, a second reseeding was made in 1935. A good stand was obtained and three cuttings were removed for each of the next 4 years.

Soil samples were taken from various layers of certain of the plots in 1931 and from all tiles in 1937 and 1939. These were taken to check on the reaction of the various layers.

In the summer of 1939, samples were taken from each soil layer from tiles representing three soil treatments to determine whether the liming had influenced the numbers of *Rhizobium meliloti* present. The samples were taken in quintuplicate by means of a soil tube. The tube was cleaned and partly sterilized by burning alcohol in it after each layer was sampled. The soil samples were then taken to the laboratory, dilutions made in sterile water, and inoculations made in triplicate to sterilized alfalfa seeds placed in sterile sand. These were then placed in the greenhouse and examined for the presence of nodules after 30 days.

As a further check on the effect of liming on root and nodule distribution, the soil was carefully removed in 8-inch layers from tiles representing three of the treatments. The soil was washed carefully to separate the roots, which were then examined for nodules. The root mass was then dried and weighed.

#### EXPERIMENTAL RESULTS

##### *Yield of alfalfa*

The average yields of alfalfa for 1930 to 1932 are given in table 3. Because of slight variations in the diameters of the tiles and possibly slight differences in compaction, some of the tiles were nearly completely filled, whereas others had several inches of space at the top. The effect of the variation in filling on yields in 1930 has been reported previously (4). Because of this, the 1930 yields have been corrected, and the corrected yields only are given. The average yields given in table 3 do not include 1930 yields, since this was the first year and only one cutting was obtained.

The data for 1930 show a marked response to the application of lime, the increase ranging from 8 to 130 per cent. Even lime in the 16-24-inch layer had a beneficial effect, as indicated by a comparison of treatments 3 and 4 with 7 and 8. Some evidence of overliming injury was obtained from the larger applications in the surface 8 inches. Direct comparisons may be made between treatments 3 and 9, 4 and 10, and 5 and 11. In each case the change from low to high rate of liming in the surface layer decreased the yields, the amounts being 315, 291, and 507 pounds, respectively. Of these, only the highest value is significant, but all indicate the same general trend.

The soil samples taken in December, 1931, showed that the pH of the surface 8-inch layer averaged 4.80, 5.85, and 7.15 for the unlimed, low-lime, and high-lime treatments, respectively. Thus, raising the pH to 7.15 with hydrated lime has apparently had a temporary depressing effect on the growth of alfalfa. This is in agreement with results obtained with other crops under greenhouse conditions (7).

The tendency toward injury from the high rates of application of lime and likewise the effect on yield of variations in the depth of soil in the different tiles largely disappeared after the first year. The results in 1931 and 1932 show a rather consistent tendency toward increases in yield for low-lime application over no lime and for the high-lime application over the low-lime. The differences, however, are not very large, the average increase in yields from the high rate of liming the surface soil over the low rate (treatments 3, 4, and 6 compared to 9, 10, and 12) being 1,877 pounds or 14 per cent. These results, as will be seen later, are quite different from those obtained during the latter years of the experiment. The relatively high yields obtained without any lime may be

TABLE 3  
*Yield of alfalfa as influenced by liming at various depths—1930-1932*

TREATMENT NUMBER	LIME APPLICATION*			ACRE YIELDS			
	0-8-inch layer	8-16-inch layer	16-24-inch layer	1930†	1931	1932	Average (1931-1932)‡
				lbs.	lbs.	lbs.	lbs.
1	None	None	None	916	7,758	15,453	11,605
2	None	High	None	990	7,925	18,831	10,878
3	Low	None	None	1,617	12,760	16,463	14,611
4	Low	Low	None	1,524	11,108	14,627	12,867
5	Low	High	None	1,669	11,842	17,350	14,596
6	Low†	None	None	1,351	10,067	15,575	12,821
7	Low	None	High	1,943	12,577	17,228	14,902
8	Low	Low	High	2,103	13,770	19,370	16,570
9	High	None	None	1,302	13,953	18,482	16,217
10	High	Low	None	1,233	13,158	15,637	14,397
11	High	High	None	1,162	13,559	17,044	15,301
12	High†	None	None	1,218	14,290	16,340	15,315
13	Low	Low	None	1,774	12,271	15,850	14,060
14	Low	Low	None	1,371	7,681	9,119	8,400

\* See table 2 for detailed treatment. Low lime applications were intended to bring the soil to approximately pH 6.0, high lime applications to approximately pH 7.0.

† One cutting only. A difference of 398 pounds is required for significance in 1930.

‡ A mean difference of 2,854 pounds is required for significance.

explained in part by the relatively high degree of base saturation in the 16-24-inch layer. Liming of this layer to high pH values resulted in an average yield increase of 1,997 pounds or 14 per cent (treatments 3 and 4 vs. 7 and 8).

The need for phosphate fertilization is clearly shown by the low yield of treatment 14 as compared to treatment 4. Potash fertilizer did not appear to have any effect on yield.

Yields for 1933 are not included in the table because the very irregular stand masked any possible effect of treatment.

The yields in 1936, 1937, 1938, and 1939, as shown in table 4, were considerably lower than for the 1931-1932 seasons. There was, however, less variation

between replicates, and the effect of liming was much more pronounced. It is quite evident that lime in all three layers had a marked effect on the yields.

The effect of liming the surface layer only may be seen by comparing the yields from treatments 1, 3, and 9. The high rate of liming increased the yields an average of 5,513 pounds yearly over that obtained from the low rate of liming and 6,887 pounds over that of the unlimed plots. Liming the surface 3 inches only was less effective than liming the surface 8 inches. This was particularly true at the high rate of liming (treatments 9 and 12). Of particular interest is the fact that the yield response obtained from liming the surface 0-8-inch layer was considerably influenced by the lime status of the lower layers. Where no

TABLE 4  
*Yields of alfalfa as influenced by liming at various depths—1936-1939*

TREATMENT NUMBER	LIME APPLICATION*			ACRE YIELDS				
	0-8-inch layer	8-16-inch layer	16-24-inch layer	1936	1937	1938	1939	Ave.†
				lbs.	lbs.	lbs.	lbs.	lbs.
1	None	None	None	1,409	1,193	1,561	1,877	1,510
2	None	High	None	3,858	4,865	5,386	4,967	4,769
3	Low	None	None	2,757	2,893	2,907	2,978	2,884
4	Low	Low	None	3,145	4,162	3,672	3,692	3,668
5	Low	High	None	6,795	7,497	7,222	8,415	7,482
6	Low‡	None	None	2,193	2,448	2,815	3,091	2,637
7	Low	None	High	6,060	7,956	9,272	10,812	8,550
8	Low	Low	High	6,451	8,476	8,629	8,129	7,921
9	High	None	None	6,121	7,313	8,629	11,526	8,397
10	High	Low	None	6,494	7,681	7,160	9,965	7,820
11	High	High	None	7,536	9,608	10,006	10,353	9,376
12	High‡	None	None	3,233	2,999	2,142	2,132	2,626
13	Low	Low	None	3,553	4,712	4,253	3,754	4,068
14	Low	Low	None	2,178	2,111	2,479	2,927	2,424

\* See table 2 for detailed explanation of lime application.

† A mean difference of 1059 pounds is required for significance.

‡ Only the surface 0-3 inches was limed in treatments 6 and 12.

lime was added to the 8-16-inch layer, the increased yield from the high over the low rate of liming of the 0-8-inch layer averaged 5,513 pounds per acre; where the 8-16-inch layer had received the low rate of liming, the corresponding increase was 4,152 pounds; and where the large amounts of lime had been added to the 8-16-inch layer, the increased yield from the heavy over the light rate of liming was only 1,894 pounds.

The effect of liming the 8-16-inch layer was also found to be influenced by the lime status of the other layers. When the surface 8 inches had received a low amount of lime, the low rate of liming the 8-16-inch layer increased the yield 784 pounds (treatment 4 over treatment 3). On the other hand, when the surface layer was heavily limed, the addition of small amounts of lime in the 8-16-inch layer had no effect on yields (treatments 9 and 10).

Heavy liming of the 8-16-inch layer increased the yield by 3,259 pounds when no lime was applied to the surface soil (treatments 1 and 2). Where the surface layer received the low amount of lime, the addition of large amounts of lime to the 8-16-inch layer increased the yield 4,598 pounds (treatments 3 and 5). Heavy liming in the 8-16-inch layer in addition to heavy liming in the 0-8-inch layer gave an increase in yield of 979 pounds, which is slightly less than the amount required for significance.

The plots receiving the high rate of liming in the 16-24-inch layer gave much higher yields than plots with similarly treated upper layers but without lime in the 16-24-inch layer. Thus, the average yield from treatments 7 and 8, where the 16-24-inch layer was heavily limed, was 5,028 pounds or 151 per cent higher than that from treatments 3 and 4, which are comparable except that this layer did not receive lime. Comparison of yields of treatments 5 and 7 indicates that the lime in the 16-24-inch layer was just as valuable as lime in the 8-16-inch layer. Actually it appears to be more valuable during the last 2 years of the experiment. The highest average yield was obtained from treatment 11 consisting of a high rate of liming to both the 0-8- and the 8-16-inch layers.

The response to phosphate fertilizer (treatment 4 vs. 14) and the failure of potash to be beneficial (treatment 4 vs. 13) are again evident.

#### *pH values after liming and downward movement of lime*

As previously noted, the pH values of samples of the surface 0-8-inch layer taken in December 1931 were 4.80, 5.85, and 7.15 for the no-lime, low-lime, and high-lime treatments, respectively. Samples representing the various layers taken from some of the tiles in 1936 showed that the pH values of the unlimed layers were between 4.9 and 5.1; the layers receiving the low rate of liming, between 5.4 and 5.7; and the heavily limed layers, about 6.6. More detailed samplings were made in 1937 and 1939.

The samples taken in 1937 showed pH values of 4.81, 5.52, and 6.45 for the no-lime, low-lime, and high-lime treatments in the surface 8 inches; 5.06 and 6.45 for the no-lime and high-lime treatments in the 8-16-inch layer; and 5.00 and 7.06 for the no-lime and high-lime treatments in the 16-24-inch layer. These values are for those samples in which the 8-inch layer immediately above was unlimed. No values are given for the low-lime treatment in the 8-16-inch layer, since in all cases the pH was influenced by downward movement from the limed layer above. In certain treatments three samples were taken representing fractions of the 8-inch layers. These showed a considerable difference between the pH values of the upper and lower part of the limed layers.

The detailed analyses of samples taken in 1939 are given in table 5. Inasmuch as each layer was sampled in three sections, it is not possible to give average values. The range for the low-lime treatment in the surface layer, for example, was from pH 5.11 to 5.85 as compared to the average of 5.52 in 1937.

The pH values of the various sections of the limed layers are of interest. In the low-lime 0-8-inch layer, the pH of the 0-3-inch section is lower in all

cases than those of the two layers below, and also that of the 6-8-inch fraction is usually higher than that of the 3-6-inch section. A similar and even more marked effect is shown in the heavily limed surface layer, in which the 6-8-inch section is more than 1 pH unit higher than the 0-3-inch section. It is evident, therefore, that there is a rather rapid downward movement of bases from the immediate surface. A similar loss of bases from the uppermost part of the heavily limed 8-16- and 16-24-inch layers is also evident.

As might be expected, there was found to be a definite movement of lime from both light and heavy applications into unlimed layers beneath. The larger the amount of lime added to the surface 0-8 inches, the greater was the downward movement into the 8-16-inch layer. This is readily evident by comparing

TABLE 5  
*Reaction of soil at various depths—1939\**

TREATMENT NUMBER	0-8-INCH LAYER			8-16-INCH LAYER			16-24-INCH LAYER		
	0-3	3-6	6-8	8-10	10-12	12-16	16-18	18-20	20-24
1	<b>4.78</b>	<b>4.82</b>	<b>4.82</b>	<b>5.01</b>	<b>5.10</b>	<b>5.05</b>	<b>4.96</b>	<b>4.96</b>	<b>5.00</b>
2	<b>4.76</b>	<b>4.75</b>	<b>4.91</b>	<b>5.83</b>	<b>6.23</b>	<b>6.36</b>	<b>5.14</b>	<b>4.96</b>	<b>4.91</b>
3	<b>5.22</b>	<b>5.80</b>	<b>5.59</b>	<b>5.23</b>	<b>5.13</b>	<b>5.05</b>	<b>4.91</b>	<b>4.93</b>	<b>4.95</b>
4	<b>5.22</b>	<b>5.68</b>	<b>5.75</b>	<b>5.77</b>	<b>5.65</b>	<b>5.24</b>	<b>4.98</b>	<b>4.94</b>	<b>4.92</b>
5	<b>5.15</b>	<b>5.53</b>	<b>5.71</b>	<b>6.16</b>	<b>6.58</b>	<b>6.79</b>	<b>5.13</b>	<b>4.90</b>	<b>4.86</b>
6†	<b>5.06</b>	<b>4.95</b>	<b>4.87</b>	<b>5.06</b>	<b>5.09</b>	<b>5.03</b>	<b>4.93</b>	<b>4.93</b>	<b>4.95</b>
7	<b>5.11</b>	<b>5.48</b>	<b>5.82</b>	<b>5.26</b>	<b>5.11</b>	<b>5.35</b>	<b>6.39</b>	<b>6.32</b>	<b>6.33</b>
8	<b>5.11</b>	<b>5.51</b>	<b>5.71</b>	<b>5.70</b>	<b>5.74</b>	<b>5.95</b>	<b>6.43</b>	<b>6.83</b>	<b>7.02</b>
9	<b>5.77</b>	<b>6.58</b>	<b>6.88</b>	<b>7.07</b>	<b>6.78</b>	<b>5.22</b>	<b>4.93</b>	<b>4.92</b>	<b>4.89</b>
10	<b>5.58</b>	<b>6.39</b>	<b>6.87</b>	<b>7.13</b>	<b>7.27</b>	<b>5.87</b>	<b>4.94</b>	<b>4.92</b>	<b>4.88</b>
11	<b>5.70</b>	<b>6.55</b>	<b>6.93</b>	<b>7.30</b>	<b>7.50</b>	<b>7.80</b>	<b>5.76</b>	<b>5.04</b>	<b>4.97</b>
12†	<b>5.71</b>	<b>5.50</b>	<b>5.01</b>	<b>5.11</b>	<b>5.15</b>	<b>5.08</b>	<b>5.01</b>	<b>5.01</b>	<b>5.00</b>
13	<b>5.28</b>	<b>5.68</b>	<b>5.79</b>	<b>5.86</b>	<b>5.84</b>	<b>5.38</b>	<b>5.09</b>	<b>5.04</b>	<b>5.02</b>
14	<b>5.32</b>	<b>5.72</b>	<b>5.85</b>	<b>5.93</b>	<b>5.89</b>	<b>5.40</b>	<b>5.05</b>	<b>5.05</b>	<b>5.02</b>

\* The values in italic type represent layers limed to approximately pH 6.0, and the values in boldface type represent layers limed to approximately pH 7.0.

† Only the surface 0-3 inches was limed in treatments 6 and 12.

treatments 1, 3, and 9. Liming the surface 0-8 inches at a rate calculated to bring the pH to 7.0 resulted in a downward movement of lime during a period of 9 years sufficient to cause greater changes in the pH values of the 8-10- and 10-12-inch sections than in that of the 0-8-inch layer to which the lime was added. The possibility of errors in sampling to exact depths is recognized, as is also the possibility of compaction or loosening of the soil, which would alter the depth of the various layers. Since the soil samples were taken carefully by means of a soil tube, however, it is highly improbable that errors in depth were more than a fraction of an inch. In addition, marked color differences between the 0-8- and 8-16-inch layers were evident, and these were used as a guide in separating these layers.

The increases in pH in the 16-18-inch section immediately below heavily

limed layers were small as compared to increases in the 8-10-inch section similarly located. This is probably explained by the fact that more rains would penetrate the 8-10- than the 16-18-inch layer.

The downward movement of lime was probably greater in this experiment than would be expected in the field, since the surface of the soil was slightly below the rim of the tile and most of the water falling on the soil was held.

#### *Root growth and nodulation*

Following the removal of the third cutting of hay in 1939 the soil from tiles representing treatments 3, 5, and 7 was removed, and the roots were carefully

TABLE 6  
*Distribution of alfalfa roots as influenced by liming*

TREATMENT	TOTAL DRY WEIGHT OF ROOTS	PERCENTAGE DISTRIBUTION OF ROOTS BY LAYERS		
		0-8-inch	8-16-inch	16-24-inch
3 (Low lime-0-0)*	gm.	per cent	per cent	per cent
	83.4	58.8	25.4	15.8
	83.5	56.9	24.5	18.5
	32.0	60.0	25.0	14.1
	Average.....	66.3	58.3	25.0
5 (Low lime-high lime-0)				
	163.6	52.0	33.1	14.9
	231.7	51.8	31.8	16.4
	147.3	59.4	28.7	11.9
	Average.....	180.5	53.9	31.4
7 (Low lime-0-high lime)				
	225.2	53.0	24.4	22.6
	162.1	55.2	22.9	21.9
	200.2	48.7	29.7	21.6
	Average.....	195.8	52.2	25.7

\* Refers to lime treatments of the 0-8-, 8-16-, and 16-24-inch layers respectively. See table 2 for further explanation.

separated from each 8-inch layer. The results of this study are given in table 6. The total dry weight of roots is related in part to the number of plants in the variously treated tiles. There were fewer plants in the tiles receiving the low-lime application to the surface 8 inches only, but even in these there was a good stand. The number of plants in the tiles that were limed in the 8-16- or 16-24-inch layer was about the same.

The relative percentage of roots in the various layers is of particular interest in indicating the effect of liming the lower layers. In all except one of the tile plots studied, over more than half of the total weight of roots was found in the surface 8 inches. The 8-16-inch layer that received lime had 31.4 per cent of

the total roots as compared to 25.0 and 25.7 per cent in the corresponding layer that was unlimed. Likewise, there were 22.1 per cent of the total roots in the 16-24-inch layer receiving lime as compared to 16.7 and 14.7 per cent in the

TABLE 7

*Number of nodules on alfalfa roots at different depths as influenced by liming*

TREATMENT	NUMBER OF NODULES		
	0-8-inch layer	8-16-inch layer	16-24-inch layer
<b>3</b> (Low lime-0-0)*	79	36	17
	13	3	2
	39	65	4
	Average.....	40	35
<b>5</b> (Low lime-high lime-0)	9	>1000	45
	2	>1000	6
	3	530	0
	Average.....	5	>1000
<b>7</b> (Low lime-0-high lime)	10	0	>1000
	0	1	>1000
	3	9	850
	Average.....	4	>1000

\* Refers to lime treatments of the 0-8-, 8-16-, and 16-24-inch layers respectively. See table 2 for further explanation.

TABLE 8  
*Influence of liming on nodule bacteria in soil*

TREATMENT NUMBER	DILUTION	AVERAGE NUMBER OF NODULES PER PLANT		
		0-8-inch layer	8-16-inch layer	16-24-inch layer
<b>3</b>	100	1.14	0.68	0.20
	1000	1.27	0.45	None
	10000	0.26	0.49	None
<b>5</b>	100	0.67	0.81	0.64
	1000	0.32	1.10	0.59
	10000	0.23	0.69	None
<b>7</b>	100	None	0.17	0.98
	1000	None	0.14	0.83
	10000	None	None	0.53

corresponding layer that was not limed. The percentage distribution was remarkably consistent for each treatment, and the differences are highly significant for the layers receiving the full lime application.

It was also noted that the small feeding roots were confined largely to the heavily limed layers. Even where the lime was applied to the 16-24-inch layer, there were mostly only large taproots in the layers above.

During the course of the root study, approximate counts were made of nodule population and distribution. The results are shown in table 7. There were relatively few nodules in the unlimed and lightly limed layers and an average of about 1000 per tile in the two layers that were limed to neutrality, regardless of whether the limed layer was at 8-16 or 16-24 inches.

These data show conclusively that the number of nodules on alfalfa roots in any soil zone is materially influenced by the lime content within that zone; moreover, it is evident that a greater abundance of nodules in one part of the root zone, resulting from a more favorable reaction or more available calcium may cause a reduction in numbers in other parts of the root system.

A further check on presence of organisms in the various layers by a dilution method similar to that employed by Wilson (11) indicated that the organisms were present in all layers. The results obtained by this method are given in table 8. The abundance of nodule organisms is indicated by the relative number of nodules produced per plant. In general, these are in agreement with the number of nodules found on the roots.

#### DISCUSSION

The threefold increase in the yield of alfalfa obtained in this investigation from the application of lime to the 16-24-inch layer shows conclusively that the degree of response to liming on a given soil cannot be predicted from studies of the surface soil only. Moreover, it emphasizes the importance of the subsurface in determining the fertility status of soils. These results are in general agreement with those obtained with fertilizer added to subsurface horizons. Lemmerman, Wiessman, and Eckl (6) found that oats and bastard clover fully utilized phosphate fertilizer incorporated between 8 and 20 inches deep. When the fertilizer was placed in the 30-40-inch layer, about half as large an increase in crop yields was obtained as when the fertilizer was applied in the 0-8- or the 8-16-inch layer. Behrens (1), working with a light sandy soil in cylinders, found that the effect of phosphate fertilizer on the yield of cereal crops decreased only slightly with depth of placement to 30 inches, but decreased sharply with deeper placement.

Liming of the 8-16-inch soil layer in these experiments increased the yield of alfalfa even when the surface 8 inches had been well limed. These results are in agreement with those obtained by Watenpaugh (9), who found that root penetration and yield of alfalfa were both materially increased when lime was applied to the 3-15-inch layer of acid DeKalb silt loam even though the 0-3-inch layer had been limed. It is evident, therefore, that the downward movement below the plow layer of lime applied to the surface should not be considered a loss but rather a process through which soil productivity can be increased.

The utilization by plants of nutrients present in subsurface horizons will, of course, be influenced by the physical characteristics of these horizons, not only because of the direct effect on root penetration but also because poor aeration

may influence nutrient absorption. It is well established, however, that the roots of many crop plants penetrate to considerable depths in many soils. Ferrant and Sprague (3) reviewed the literature on the effect of various soil conditions on root development and penetration. In their own investigations they found that the root distribution of red clover in Sassafras loam in New Jersey was much less extensive than had been reported for soils in the western and midwestern states. Tillage of the A<sub>2</sub> horizon resulted in a greatly increased penetration of the red clover roots into the B horizon.

As shown in this investigation, however, the chemical properties of the subsurface soil may materially influence root penetration where the physical condition of the soil is favorable to such penetration. Thus, the proportion of the total roots in the 16-24-inch layer was found to be about 50 per cent greater where this layer had been limed to about neutrality than where only the surface 0-8 inches or the 0-8- and the 8-16-inch layer had been limed. Moreover, the fibrous roots were largely concentrated at the 16-24-inch depth where this layer had received the lime. This is in agreement with the results reported on the fertilization of subsurface soil horizons (8, 10). It is evident, therefore, that under certain conditions the deep placement of lime or fertilizers may be an advantage in that it results in deeper root penetration and greater absorption of water or other nutrients by the plants. According to Sokolav (8) the moisture content of the subsurface horizons has less influence on root distribution than has the amount of nutrient elements present.

One of the most striking results obtained in this investigation was the effect of liming the subsurface layers on the distribution of the nodules in the root system. Where the 16-24-inch layer was limed to about neutrality the nodules were largely concentrated in this layer. Similarly, most of the nodules occurred in the 8-16-inch layer where the lime had been applied to this layer. It is apparent that nodule organisms of alfalfa cannot function properly in acid soil layers even though the plant obtains all its calcium needs from other parts of the root system. This direct relationship between nodulation and lime content is in accordance with the results obtained by Bryan (2) and by Karraker (5) in greenhouse experiments.

#### SUMMARY

Alfalfa was grown in large glazed tiles filled with three layers, each 8 inches deep, of Gilpin silt loam variously limed before being placed in the tiles. Two rates of liming were studied, one calculated to bring the soil to pH 6.0, the other to pH 7.0. Yields of alfalfa were obtained for two 4-year periods. Soil samples were taken at various depths during the course of the experiment, and detailed samples were taken for studies of downward movement of lime 9 years after application. At the conclusion of the experiment the effect of lime on root distribution and on nodulation was determined from several of the treatments. The major results may be briefly summarized as follows:

The effect of different liming of the three soil layers was more pronounced during the last 4 years of the experiment than during the first 4 years, probably because of the effect of soil disturbance during the first few years.

Liming of the surface 0-8 inches to a pH of approximately 7.0 resulted in a depression in yield during the first year of the experiment.

Liming the 16-24-inch layer to approximate neutrality more than tripled the yield of alfalfa when the surface 0-8 inches had a pH of about 5.6. Approximately the same increases in crop yields were obtained as when either the 0-8- or 8-16-inch layer was similarly limed.

The liming of the 8-16-inch layer to approximately pH 6.0 tripled the yield of alfalfa when no lime had been added to the surface 0-8-inch layer.

Maximum yields were obtained where both the 0-8- and the 8-16-inch layers were limed approximately to neutrality.

The increases in yield obtained from raising the pH of the surface soil from about 5.6 to 6.5 were dependent on the lime status of subsurface horizons. Where the 8-16-inch layer received no lime, the increase in yield from heavy liming of the surface was 5,513 pounds per acre; where the 8-16-inch layer was limed to about 5.6, the increase was 4,152 pounds; and where it had been limed to about neutrality, the increase was only 1,894 pounds per acre.

A marked downward movement of lime occurred not only from one layer to another but also within the layer receiving the lime, the difference in reaction between the 0-3- and the 6-8-inch sections after 9 years being over 1 pH unit.

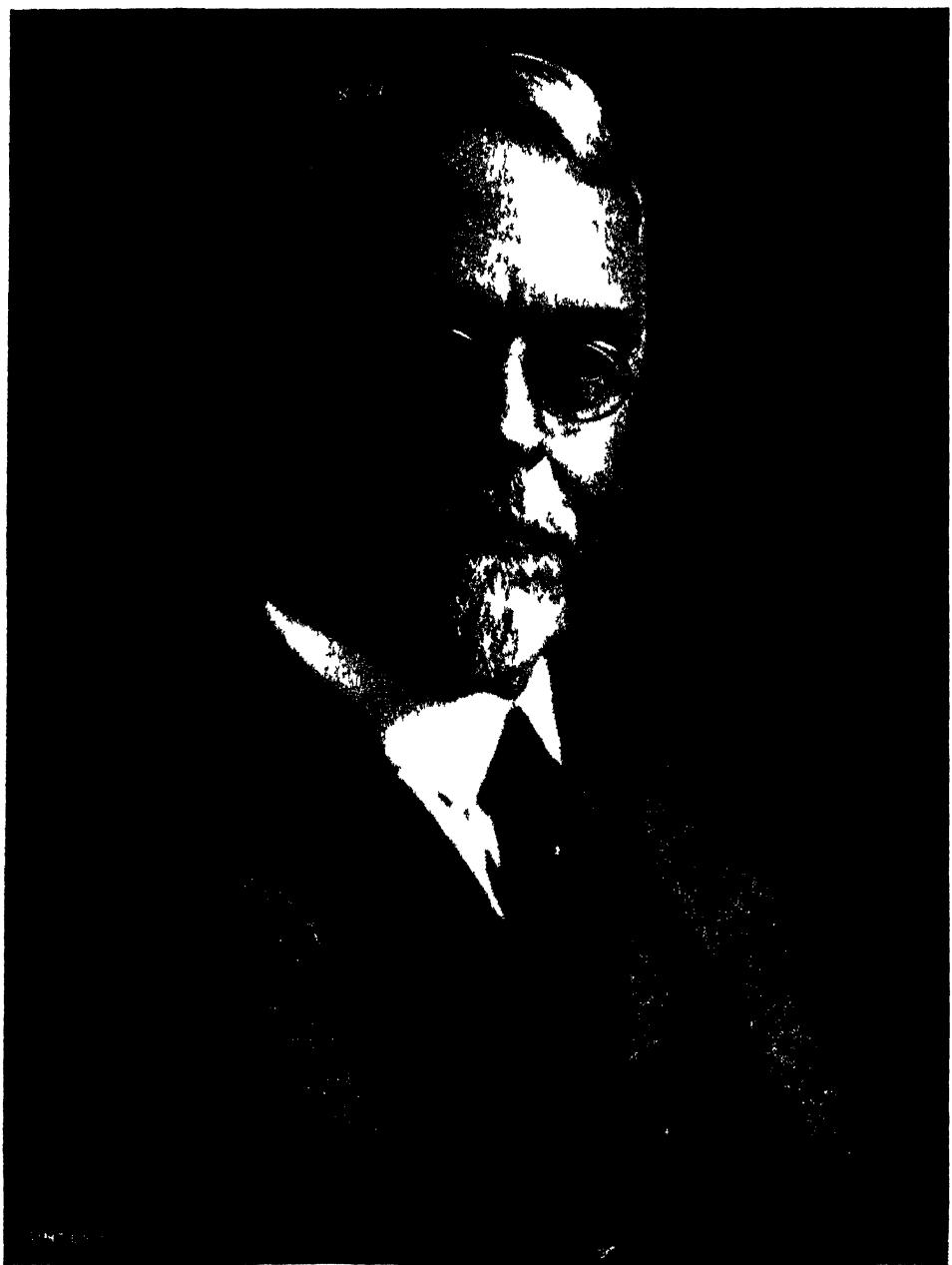
Root distribution was markedly affected by liming of the subsurface horizons. Liming the 16-24-inch layer to about neutrality resulted in an increase of 50 per cent in the proportion of the total root growth found in this layer. Moreover, the fibrous roots were largely concentrated in this layer.

The nodules on the roots were largely concentrated in the layer receiving the high-lime treatment, irrespective of whether this was the 8-16- or the 16-24-inch layer.

#### REFERENCES

- (1) BEHRENS, W. U. 1934 Die Aufnahme und Verwertung der Phosphorsäure aus den verschiedenen tiefen Bodenschichten durch die Pflanzen. *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* 36A: 343-348.
- (2) BRYAN, O. C. 1923 Effect of acid soils on nodule-forming bacteria. *Soil Sci.* 20: 37-40.
- (3) FERRANT, N. A., JR., AND SPRAGUE, H. B. 1940 Effect of treating different horizons of Sassafras loam on root development of red clover. *Soil Sci.* 50: 141-161.
- (4) GARBER, R. J., AND PIERRE, W. H. 1933 Variation of yields obtained in small artificially constructed field plots. *Jour. Amer. Soc. Agron.* 25: 98-105.
- (5) KARRAKER, P. E. 1927 Production of nodules on different parts of the root systems of alfalfa plants growing in soils of different reaction. *Soil Sci.* 24: 103-107.
- (6) LEMMERMANN, O., WIESSMAN, H., AND ECKL, K. 1925 From what depth in the soil can plants usefully obtain nutrients? *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* 4: 233-241. [Abstract in *Chem. Abs.* 20: 1127.]
- (7) PIERRE, W. H., AND BROWNING, G. M. 1935 The temporary injurious effect of excessive liming of acid soils and its relation to the phosphorus nutrition of plants. *Jour. Amer. Soc. Agron.* 27: 742-759.
- (8) SOKOLAV, A. V. 1937 Effect of the distribution of nutritive elements and moisture at different levels in the soil on root development and yield of plants. *Trans. Sci. Inst. Fert. Insectofungicides (U.S.S.R.)* 136: 203-417. [Abstract in *Chem. Abs.* 33: 1858.]
- (9) WATENPAUGH, H. N. 1936 The influence of the reaction of soil strata upon the root development of alfalfa. *Soil Sci.* 41: 449-468.
- (10) WEAVER, J. E., JEAN, F. C., AND CHRIST, J. W. 1922 Development and activities of roots of crop plants. *Carnegie Inst. Wash. Pub.* 316.
- (11) WILSON, J. K. 1926 Legume bacteria population of the soil. *Jour. Amer. Soc. Agron.* 18: 911-919.





Homer J. Wheeler  
1861-1945

## Homer J. Wheeler

1861-1945

Doctor Homer Jay Wheeler, pioneer agricultural chemist and agronomist, died at his home in Upper Montclair, New Jersey, November 18, 1945.

Born at Bolton, Massachusetts, September 2, 1861, he lived in that state until after his graduation from Massachusetts Agricultural College and Boston University in 1883, and for a training period of 4 years as assistant chemist at the Massachusetts Experiment Station. With the encouragement of Dr. Goessman, chemist and director of that station, he matriculated at Göttingen University and received the degree of doctor of philosophy in 1889. While at Göttingen he was invited to become one of the five workers on the staff of the newly organized Rhode Island Experiment Station. He began his long service there on September 1, 1889, as chemist, undertook additional responsibility as agronomist in 1897, and became the third director in 1900. He was also professor of agricultural chemistry and of geology during much of the period of his directorate and was acting president of the college for an interval of several months. He became manager of the Agricultural Service Bureau of the American Agricultural Chemical Company in 1912, and served in that capacity until 1931, when he resigned to become a free-lance agricultural consultant.

During his stay in Germany, Dr. Wheeler met Frieda Ruprecht, who became his wife in 1891. She survives him with their three sons, Carl, William, and Roland.

Dr. Wheeler entered upon his long career with the new research organization at Rhode Island with the energy and enthusiasm that characterized his activities throughout his life. Before leaving Göttingen, he received a grant of \$2,000 to purchase equipment for a new chemical laboratory at Rhode Island. He obtained 250 books and bound volumes of scientific journals for \$500, and with the remainder he bought "a polarizing microscope, a polarimeter, 3 analytical balances, a combustion furnace, platinum dishes and crucibles, 150 reagent bottles, a large stock of filter paper, thermometers, hydrometers, about 1300 miscellaneous pieces of laboratory glassware, besides wooden and iron ware and the necessary supply of porcelain." Obtained without payment of duties and dealers' profits, this was considered remarkable economy, even in that day of uninflated prices. Some of the items are still in use at the Station. As further preparation for his work, he visited experimental stations at Zurich, Darmstadt, Bonn, Hildesheim, Bernberg, Möckern, Halle, Berlin, and Rothamsted and various phosphate works and potash mines in Germany.

During his first year as chemist he wrote the first technical bulletin published from the Rhode Island Station, prepared a file index of all experiment station bulletins published in the country at that time, organized a temporary laboratory in a near-by city, assumed active charge of cooperative fertilizer experiments at ten places in the state, surveyed the first experimental plats at the Station Farm, made detailed chemical analyses of varied fertilizer materials, well water, and sewage, and investigated the fraudulent sale of linseed meal as a dyestuff.

Of his subsequent work at Rhode Island, his contemporary, Dr. E. W. Allen, of the Office of Experiment Stations, wrote the following statement of appreciation in 1929:

He instituted a system of laboratory, pot, and field experiments which in connection with chemical studies resulted in outstanding contributions to the knowledge of soil fertility, notably the nature, cause, and cure of so-called acid soils, which he showed to be widespread in this country, and the effect of one crop on another in rotation. He studied intimately the action of various fertilizing materials, the nutritive requirements of plants and methods of determining these needs, sodium salts and their functions in soils and plants, the relations of lime and magnesia to plant growth, and the effect of the soil reaction on various crops and on the prevalence of such diseases as potato scab. In that connection, he contributed to the differentiation of acid-tolerant crops from those unsuited to acid soils and showed the relation of soil treatment to acidity. He was the first to note the effect of sulfate of ammonia in increasing the acidity of soils deficient in lime.

Much of the work was done in close collaboration with the late Dr. Burt L. Hartwell, who succeeded Dr. Wheeler as station chemist in 1908 and who continued the same lines of work for many years after becoming director in 1913. Some of the experiments are still in operation on the original plats.

Dr. Wheeler was proud of the accomplishments of the Station and expended much time and energy in explaining practical applications of its work to the farmers of the state. As the small agricultural school grew to Rhode Island State College, he worked equally hard to advance its growth, and to prevent its encroachment on the financial support of the Station. After taking a vigorous part in a controversy with Brown University concerning allocation of the Federal Land-Grant Funds, he was gratified when that University recognized his services to agriculture by conferring upon him the degree of doctor of science. Impressed by the need for adult education for the farm population, he was an early advocate of the Agricultural Extension Service.

As manager of the Service Bureau of the American Agricultural Chemical Company, Dr. Wheeler extended his efforts from the small area of Rhode Island to the states of the Atlantic Coast, the Corn Belt, and to California. He organized a staff of agronomists and trained photographers to conduct literally hundreds of experiments with mixed fertilizers and superphosphates on farms in the eastern, southern, and middle western states. These investigations ranged from potatoes in Maine to citrus in Florida, and from cotton in the Southern states to field crops in the fertilizer-frontier sections of the middle west. This early and extensive use of field experiments on the farm led the way to a greater use of this demonstrational method in agriculture. He was among the first to recognize the need for phosphorus, and ultimately for other fertilizer elements, in the great midwestern Corn Belt. It is interesting that while others thought that the first need for phosphorus might be in the less fertile areas, he recognized signs of deficiency in the crops of the fertile Red River Valley of Minnesota, now classified as one of the phosphorus-deficient areas of the country.

From his early studies in Rhode Island, he foresaw the need for magnesium in fertilizers long before crop failures in many eastern states demonstrated the deficiency. When these first occurred in Aroostook County, Maine, crops grown

with the fertilizer formula that he had designed continued to produce excellent yields. He was also among the first to experiment with fertilization of pastures, for in 1930 he published the results of 109 demonstrations made on hay and pasture lands of 18 states and in Canada. This book, *Facts About Pasture Fertilization and Mineral Deficiency*, still ranks as a valuable reference.

In 1913, Dr. Wheeler published the widely used text, *Manures and Fertilizers*. Later he wrote more than 20 booklets, which included such variety as: How to Select and Apply Fertilizers; How to Get the Most out of Fertilizers; Citrus Profits; Golf Course Maintenance; Some Essential Points in Tobacco Culture; Profitable Fertilizers for California Crops, and Citrus Culture in California. As an employee of industry, he guarded jealously a reputation for unbiased scientific observation and judgment, and was quick to resent inferences to the contrary, on behalf of himself and his colleagues.

Active in many agricultural organizations, he was elected to the presidency of the Association of Official Agricultural Chemists in 1902 and of the American Society of Agronomy in 1911. He was awarded the degree of doctor of science by Brown University in 1911 and by Massachusetts State College in 1933.

A brilliant student, Dr. Wheeler sought always to advance agriculture through scientific knowledge. He supported loyally those with the optimism and imagination to foresee improvement, but scorned the pessimist who resisted change. His long career brought honor to his memory and progress to his country.

JOHN B. SMITH.



# THE GENESIS OF STRUCTURE IN TWO CALCAREOUS SOILS<sup>1</sup>

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The mechanism of the genesis of soil structure has been the basis of numerous investigations. Nevertheless, there is still considerable disagreement concerning the nature of this mechanism. The experiments here presented were designed to study the relation of microorganisms and of roots to the formation of water-stable soil structures.

As early as 1916, Waksman (14) suggested that "fungal mycelia may act as binding agents for soil granules"; but in 1940, when Baver (1) summarized the literature, the generally accepted theory of aggregate formation was that of cementation or of cohesion of the soil colloids. Since 1940 the role of microorganisms in the genesis of soil aggregates has received considerable attention (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15). Martin and Waksman (7) first demonstrated that pure and mixed cultures of microorganisms, when introduced into sterilized soil, increase aggregation. Waksman (14), Peele (12), and Martin and Waksman (7) found that the presence of microorganisms in soil augmented aggregate formation and retarded erosion. Martin (9) concluded that as much as 80 per cent of the effect of microorganisms on soil aggregation was attributable to the cementation action of cell secretions.

In the present studies, Gila clay and Tucumcari sandy loam soils were used. These two soils differ widely in texture, but both are calcareous. Some physical and chemical properties of the two soils follow:

SOIL	MECHANICAL ANALYSES			pH	TOTAL SOLUBLE SALTS per cent	CALCIUM CARBONATE per cent	ORGANIC MATTER per cent
	Sand per cent	Silt per cent	Clay per cent				
Gila clay.....	24.5	43.2	32.3	7.60	0.14	7.56	1.70
Tucumcari sandy loam.....	60.1	20.7	19.2	7.55	0.07	0.26	1.45

## METHODS

To destroy existing structure above 0.25 mm. in diameter, the soils were passed through an 80-mesh sieve. One-hundred-gram portions were weighed into 1,000-ml. Erlenmyer flasks and sterilized by heating in the dry state for 24 hours at a temperature of 300° C. After sterilization the clay soil was brought to a moisture content of 40 per cent, and the sandy loam soil to one of 30 per cent by the addition of sterile soil extract, sterile nutrient medium, or sterile distilled water, depending on the intended use. In the liquid nutrient-medium set,

<sup>1</sup> Contribution of the Soil Conservation Service, U. S. Department of Agriculture, in co-operation with department of agronomy, New Mexico Agricultural Experiment Station, State College, New Mexico.

those destined for inoculation with molds received Fred and Waksman's (2) medium No. 16; with actinomycetes, No. 36; with bacteria, No. 5. The flasks were plugged with cotton and resterilized in an autoclave at 20 pounds' pressure for 4 hours. These prolonged periods of sterilization probably resulted in loss of some organic matter and in other changes; they were, however, found necessary for complete sterilization, which was of paramount importance.

Cultures of fungi, actinomycetes, and bacteria were isolated from the original soils by plating on soil-extract agar. No attempt was made to separate the species in any of these groups, but the groups were kept separate. Liquid cultures of each of the three groups were made from the isolates. After 30 days' incubation, these cultures were used for inoculating each of the two soils in duplicate to determine the effects of the three groups of organisms on the formation of aggregates. The inoculum for each flask constituted a 10-ml. portion of the appropriate liquid culture. Three sorts of controls were used: one to which sterile soil extract was added, another that received a like amount of sterile distilled water, and a third, comprising three duplicate cultures, each of which received one of the liquid media.

The remainders of the liquid cultures from which the inocula had been taken were divided into two parts and sterilized, one part by steaming for 1 hour on each of five consecutive days and the other by filtering through a number 9 Mandler serum filter. The resulting liquids were used in a series of flasks of the two soils to compare the effects of the living organisms with those of their products of secretion or of decomposition. Preparation of these treatments differed slightly from the ones previously described: to a flask containing 100 gm. of sterilized dry soil, a portion of one of the six liquids was added. In the clay the amount added was 50 ml.; in the sandy loam, 40 ml. Thus the moisture content of all treatments was the same within each soil. The cultures were incubated at 26° C. for 21 days.

To determine the role of roots in aggregate formation, a set of flasks similar to those previously described were planted with 50 sudan grass (*Sorghum vulgare* var. *sudanense*) seeds each. These cultures were inoculated with a mixture of the three groups of organisms. Duplicate uninoculated cultures in each soil served as controls. The seeds were sterilized by shaking for 5 minutes in 0.25 per cent  $HgCl_2$ , and then soaking for 3 hours in 26 per cent chlorinated lime solution. This method of sterilization proved unsatisfactory, and later sets were treated under reduced pressure for 40 minutes in a 0.25 per cent solution of  $HgCl_2$  and were not soaked in the chlorinated lime solution. The flasks of this set were incubated at room temperature for 18 days. Another set of sudan grass cultures in pulverized but unsterilized soil were planted with unsterilized seed. The controls of this set were of similarly treated soil without the grass.

Aggregation was determined by wet-sieving the soil from the flasks for 30 minutes at 20 strokes per minute. The amplitude of the stroke was approximately 2 inches. The sieve openings were 0.25, 0.59, 1.19, 2.00, and 4.76 mm. The aggregates on the different screens were collected, dried at 95° C., and weighed. Aggregates from each flask of the inoculated treatments were exam-

ined microscopically to ensure absence of the other two groups of organisms; those of the uninoculated treatments, to determine contamination. When contamination of either group was detected, the treatment was repeated in duplicate, but contamination was not assumed unless the organisms were actually seen under the microscope. Because of the difficulty of obtaining complete sterilization of the soil and of the seed, many repetitions were necessary. As presented in the tables, the data comprise results from at least four uncontaminated flasks. Samples from the field were observed for comparison with the laboratory cultures.

TABLE 1  
*Average aggregating effect of microorganisms and of microbial products on two calcareous soils*

SOIL	TREATMENT	AGGREGATION				
		2.00 mm. per cent	1.19 mm. per cent	0.59 mm. per cent	0.25 mm. per cent	Total per cent
Gila.....	Sterile distilled water	0.00	0.00	0.00	0.00	0.00
Tucumcari.....	Sterile distilled water	0.00	0.00	0.00	0.00	0.00
Gila.....	Sterile soil extract	0.00	0.00	0.00	0.00	0.00
Tucumcari.....	Sterile soil extract	0.00	0.00	0.00	0.00	0.00
Gila.....	Sterile media	0.00	0.00	0.00	0.00	0.00
Tucumcari.....	Sterile media	0.00	0.00	0.00	0.00	0.00
Gila.....	Bacteria	0.00	0.00	0.09	0.15	0.24
Tucumcari.....	Bacteria	0.00	0.00	0.07	0.12	0.19
Gila.....	Actinomycetes	1.07	0.61	0.80	0.88	3.36
Tucumcari.....	Actinomycetes	1.27	0.76	0.91	0.83	3.77
Gila.....	Fungi	1.59	0.77	0.77	0.56	3.69
Tucumcari.....	Fungi	2.57	0.32	0.24	0.14	3.27
Gila.....	Bacterial products	0.00	0.00	0.00	0.00	0.00
Tucumcari.....	Bacterial products	0.00	0.00	0.00	0.00	0.00
Gila.....	Actinomycete products	0.00	0.00	0.00	0.00	0.00
Tucumcari.....	Actinomycete products	0.00	0.00	0.00	0.00	0.00
Gila.....	Fungal products	0.00	0.00	0.00	0.00	0.00
Tucumcari.....	Fungal products	0.00	0.00	0.00	0.00	0.00

## RESULTS

The results indicate that there were two sorts of water-stable structures present in the soil of the seedling cultures and in that from the field: microbial structures comprising soil particles, or aggregates, held together by microorganisms, and root structures, or compound aggregates, comprising microbial aggregates and entrapped soil particles held together by roots. Conceivably, fungal hyphae may bind together simple aggregates to form small, water-stable, compound aggregates which, logically, differ from those formed by roots only in the strength of the binding agent. Actually, however, water-stable fungal structures—whether simple or compound—have not been observed with sizes greater than 5 mm.—the observed upper limit of simple fungal aggregates. Though struc-

tures held together by roots may be smaller than this, they have, in all observed cases, been composed of microbial aggregates with or without entrapped soil particles. For this reason and pending further investigation, the microbial structures will be referred to as *aggregates* and the structures containing roots as *root-structures*.

The data presented in table 1 show that fungi, actinomycetes, and bacteria all produced aggregates in both Gila clay and Tucumcari sandy loam, no consistent difference being apparent between the two soils. Aggregation was much less in the bacterial cultures than in those of fungi or of actinomycetes. The addition

TABLE 2

*Effect of microorganisms on aggregation with an added source of energy*

SOIL	TREATMENT		AGGREGATION					
	Energy source	Inoculum	2.00	1.19	0.59	0.25	Total	
			mm.	mm.	mm.	mm.		
Gila.....	Sterile media	.....	0.00	0.00	0.00	0.00	0.00	
Tucumcari....	Sterile media	.....	0.00	0.00	0.00	0.00	0.00	
Gila.....	Sterile soil extract	Bacteria	0.00	0.00	0.08	0.10	0.18	
Gila.....	Sterile soil extract plus medium	Bacteria	0.00	0.00	0.05	0.08	0.13	
Tucumcari....	Sterile soil extract	Bacteria	0.00	0.00	0.08	0.10	0.18	
Tucumcari....	Sterile soil extract plus medium	Bacteria	0.00	0.00	0.03	0.04	0.07	
Gila.....	Sterile soil extract	Fungi	0.27	0.16	0.13	0.12	0.68	
Gila.....	Sterile soil extract plus medium	Fungi	2.92	1.29	1.20	1.01	6.42	
Tucumcari....	Sterile soil extract	Fungi	0.23	0.19	0.18	0.15	0.75	
Tucumcari....	Sterile soil extract plus medium	Fungi	4.92	0.46	0.31	0.13	5.82	
Gila.....	Sterile soil extract	Actinomycetes	0.62	0.33	0.79	1.23	2.97	
Gila.....	Sterile soil extract plus medium	Actinomycetes	1.53	0.89	0.81	0.54	3.77	
Tucumcari....	Sterile soil extract	Actinomycetes	0.95	0.76	0.89	1.07	3.67	
Tucumcari....	Sterile soil extract plus medium	Actinomycetes	1.59	0.76	0.94	0.60	3.89	

of a source of carbon caused a marked increase in aggregation in the cultures of fungi and of actinomycetes but a decrease in those of bacteria (table 2).

No aggregates were produced in any of the sterile treatments, unless the cultures were contaminated by living organisms. In every case where aggregation occurred, including those treated with the sterilized cultures, living organisms were observed when the aggregates were crushed and examined under the microscope.

The role of roots in the synthesis of soil structure was less easily demonstrated in culture. Under aseptic conditions, the seeds failed to germinate, or the size of the root systems was almost negligible (table 3). Germination occurred in only seven of more than 50 sterile cultures, and in these the root systems were

very weak. In these seven cultures, the average germination was 14 per cent. Compared to that in unsterilized or in inoculated soil, where germination amounting to 85 per cent occurred within 24 hours, there was a delay of 3 to 5 days. Johnson (3), working with a large number of species on soils heated at temperatures ranging from 50° to 800° C., found temporary retardation of germination

TABLE 3  
*Effect of sudan grass roots and mechanical treatment on aggregation*

SOIL	TREATMENT	ROOTS	ROOT STRUCTURES ABOVE 4.76 MM	AGGREGATION						Total
				4.76 mm.	2.00 mm.	1.19 mm.	0.59 mm.	0.25 mm.	-	
				gm.	per cent	per cent	per cent	per cent	per cent	
Gila	Untreated field sample	0.21	10.00		4.31	5.79	4.86	9.73	24.69	
Tucumcari	Untreated field sample	0.51	63.39		7.69	4.45	6.45	8.39	27.18	
Gila	Pulverized soil			0.00	3.17	0.87	0.80	1.99	6.83	
Tucumcari	Pulverized soil			0.00	0.34	0.14	0.22	5.93	6.63	
Gila	Pulverized sterilized soil*			0.00	0.16	0.25	0.43	0.20	1.04	
Tucumcari	Pulverized sterilized soil*			0.00	0.08	0.08	0.08	0.26	0.50	
Gila	Pulverized sterilized soil†			0.00	0.00	0.00	0.00	0.00	0.00	
Tucumcari	Pulverized sterilized soil†			0.00	0.00	0.00	0.00	0.00	0.00	
Gila	Pulverized sterilized soil, inoculated			0.00	2.74	0.63	0.45	0.50	4.32	
Tucumcari	Pulverized sterilized soil, inoculated			0.00	2.65	0.13	0.20	0.22	3.20	
Gila	Pulverized soil plus roots	1.38	8.21		2.80	0.63	0.66	2.28	6.37	
Tucumcari	Pulverized soil plus roots	1.60	34.68		1.77	1.46	0.48	2.59	6.30	
Gila	Pulverized sterilized soil* plus roots	0.34	0.00	0.00	0.00	0.26	0.25	0.40	0.91	
Tucumcari	Pulverized sterilized soil* plus roots	0.47	0.00	0.00	0.15	0.07	0.11	0.15	0.48	
Gila	Pulverized sterilized soil† plus roots	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Tucumcari	Pulverized sterilized soil† plus roots	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Gila	Pulverized sterilized soil, inoculated, plus roots	0.55	1.80	0.00	1.33	0.66	0.67	0.68	3.34	
Tucumcari	Pulverized sterilized soil, inoculated, plus roots	0.51	4.08	0.00	0.78	0.64	0.45	0.36	2.23	

\* Grown in an open beaker for 21 days under laboratory conditions.

† Grown for 21 days under sterile conditions.

and poor growth on the soils heated to temperatures of 100° to 350° C. These effects were more marked when sterile conditions were maintained. Johnson's description (3, p. 33) of growth of roots of lettuce germinated and allowed to grow on the surface of heated soil applies equally well to the growth of the plants in the sterile cultures of the present experiments:

. . . the roots instead of penetrating the soil, grow along the surface of the soil. When the roots are, however, forced to grow into certain sterilized soils in order to maintain the life of the plant, the roots may be much retarded in growth and may become short and stubby, without root hairs, frequently discolored as a whole or in local areas, deeply split radially,

partly decayed and sometimes entirely killed. This is especially true . under sterile conditions.

In unsterilized, contaminated, or inoculated cultures germination and root development were good, and there was no observable difference in the growth of plants in these three types of cultures. Where contamination was slight, only a minute amount of aggregation occurred, and the roots did not enter into the process; where there was no contamination, no aggregation was observed even in

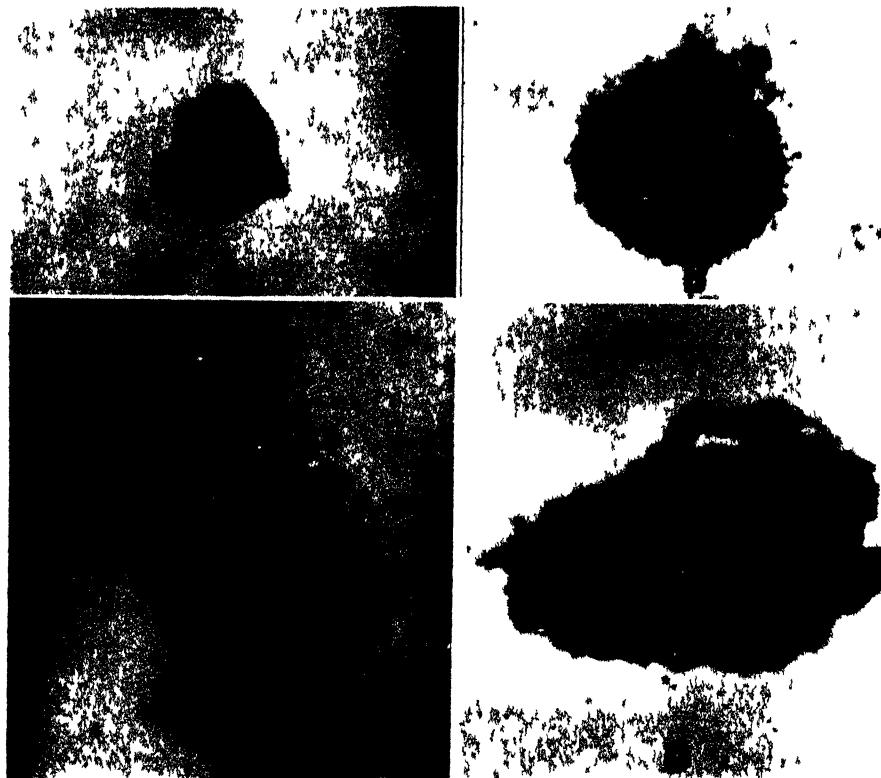


FIG. 1. COMPARISON OF FOUR TYPES OF AGGREGATES

A, (Upper left) 0.25 mm bacterial,  $\times 40$ , B, (Upper right) 0.84 mm actinomycete,  $\times 19$ , C, (Lower left) 2.00 mm fungal,  $\times 20$ , D, (Lower right) 0.58 mm normal soil  $\times 17.5$

the seven cultures in which there was some growth of roots. In inoculated cultures and in untreated soils, aggregate and root-structure formation was active (table 3). The presence of roots in the cultures did not affect the total amount of aggregation, but it increased the size of the aggregates. Because of the brief period of growth, perhaps, the level of aggregation in the cultures was lower than that observed in field samples.

Each group of microorganisms produced an easily recognizable type of aggregate. These are illustrated in figure 1.

Figure 1A shows an aggregate formed by bacteria. Structures of this type are small, compact, and commonly angular. The edges and surfaces are smooth. Although they are water-stable, these aggregates are fragile and easily crushed. The maximum size observed was 0.56 mm.

Figure 1B presents an aggregate formed by actinomycetes. This type is resistant to crushing, fairly compact, not angular, and, under the microscope, decidedly fuzzy on the surface. To the unaided eye, the representatives of this type are hardly distinguishable from those formed by bacteria, except that they may attain diameters of 2 mm.

In figure 1C are shown aggregates formed by fungal hyphae. The spongy masses of this type are rarely less than 2 mm. in diameter and often attain diameters greater than 4.76 mm. They are very resistant to crushing. In aggregates with diameters of less than 2 mm., much of the soil material washes

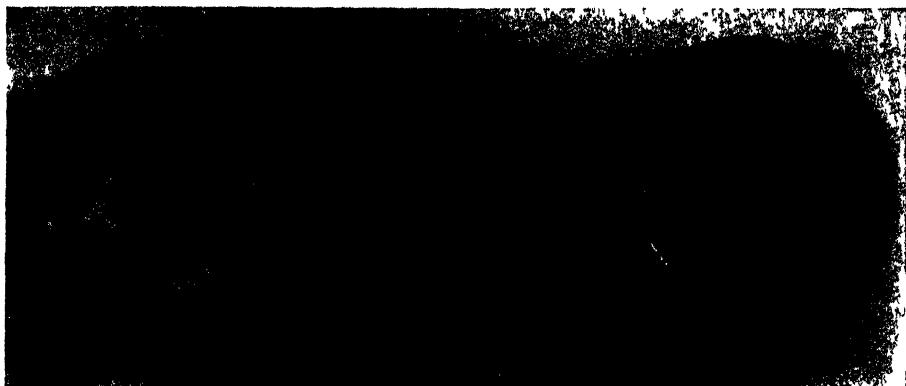


FIG. 2. THREE TYPES OF AGGREGATES SUBMERGED IN WATER  
A, fungal; B, actinomycete; C, bacterial

out of the mycelial framework, and the aggregates do not hold their typical form.

When the different types of aggregates were placed in water, the bacterial aggregates remained dispersed as separate units, the actinomycete aggregates tended to become grouped into small clumps, and those of fungal origin became massed so that it was impossible to distinguish the individuals (fig. 2).

Samples from the field gave results that were in accord with those from the laboratory cultures (fig. 1D). Stable soil structure in the field samples was always associated with a good growth of roots and microorganisms. Wet-sieving of such a soil resulted in a relatively high proportion of water-stable structures, ranging in diameter from 0.25 mm. to approximately 3 cm. Those larger than 0.6 mm. were usually held together by roots, the size and number of the roots varying with the size of the structure. The smaller aggregates—less than 0.6 mm. in diameter—when crushed and teased with a needle under magnification, tended to follow the needle in the manner of a tuft of cotton. The soil granules making up these aggregates were entrapped among interlaced fungal



A



B

FIG. 3. A, A CRUSHED TUCUMCARI SANDY LOAM AGGREGATE, SHOWING THE SOIL GRANULES ENMESHED IN A TANGLE OF FUNGAL HYPHAE,  $\times 92$ ; B, FUNGAL HYPHAE AND SOIL GRANULES FROM A PORTION OF A CRUSHED AGGREGATE,  $\times 155$

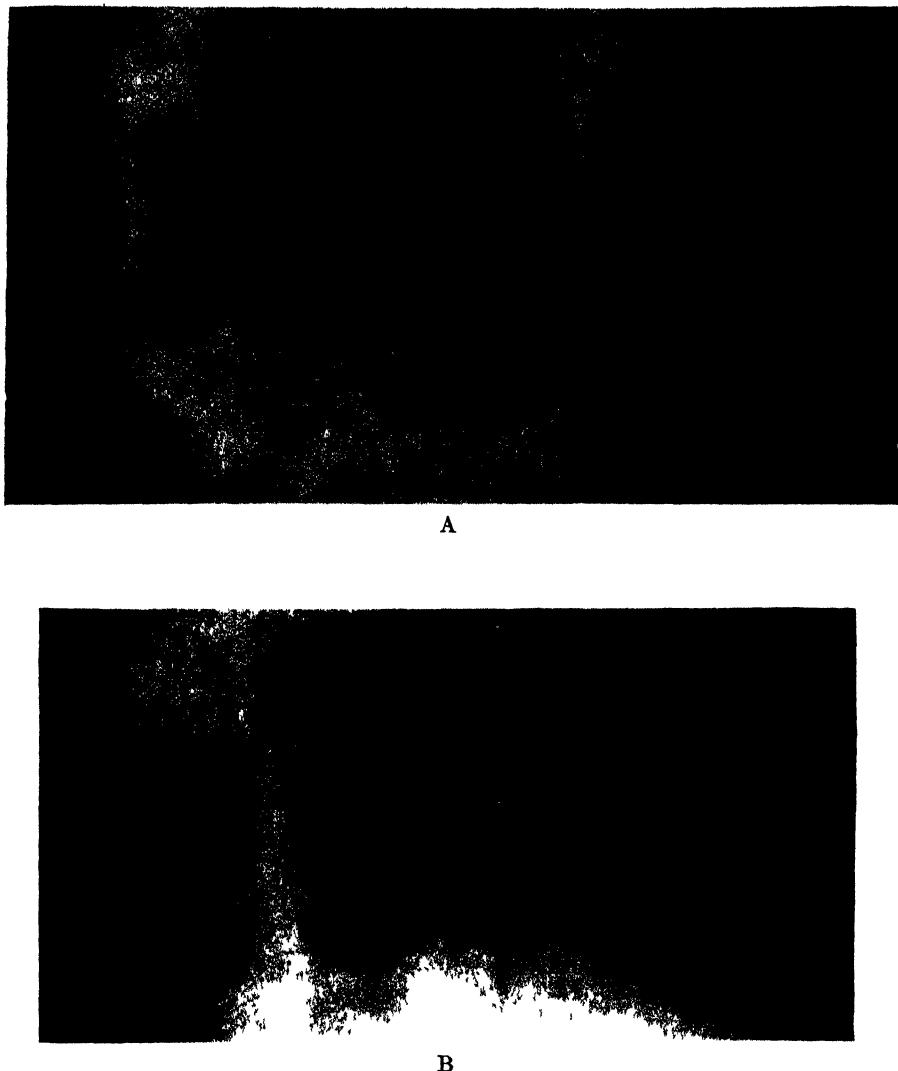


FIG. 4. SEQUENCE IN THE FORMATION OF SOIL STRUCTURES: A, (UPPER) AGGREGATES, VARYING IN DIAMETER FROM 0.25 TO 0.60 MM., AND A ROOT STRUCTURE,  $\times 17.5$ ; B, (LOWER) STAGES IN THE GROWTH OF A ROOT STRUCTURE,  $\times 17.5$

hyphae (fig. 3). Since actinomycetes and bacteria as well as fungal hyphae entered into the make-up of these structures, their appearance was not so characteristic as that of the ones obtained in laboratory cultures: they were neither so spongy as the pure fungal aggregates nor so smooth as those resulting from bacterial growth. Moving pictures were taken to illustrate these findings.<sup>2</sup>

<sup>2</sup> Chapman, J. E., and Hubbell, D. S. 1945 The evolution of soil structure. U. S. Dept. Agr. Soil Conservation Research—16 mm. silent movie film.

Several aggregates and a small root structure, all isolated from the Gila soil as it came from the field, are illustrated in figure 4A. Figure 4B represents three stages in the growth of a root structure.

#### DISCUSSION

The results of the present studies do not bear out the conclusion of Martin (9) that the main effect of microorganisms on aggregation is attributable to cell secretions as such. Though the cell products in conjunction with the secreting cell constitute an important factor in aggregate formation by all three groups of organisms (soil particles were observed clinging tenaciously to filaments of molds and actinomycetes), no aggregation was observed when soil in which the structure had been destroyed was treated with liquid media in which organisms had grown for 30 days and then had been removed or destroyed. Water-stable aggregates were never observed except in the presence of living microbes.

Each group of microorganisms was capable of initiating aggregation; roots, on the contrary, contributed to structure formation only after this had been initiated microbially, and microorganisms were found in every root structure that was examined microscopically. The root structure seems to be made up of numerous microbial structures held together by the root or root mass. It is probable that roots may contribute to the growth of the microbes in the immediate neighborhood and in this way expedite structure formation. It is possible, too, that the organisms may affect the mineral nutrition of the plant beneficially. This might explain the poor growth noted in the field with loss of soil structure. The augmenting of aggregation by the use of an added source of carbon to mold and actinomycete cultures is in accord with the results of Peele and Beale (13), who obtained similar results with several different sources of added carbon.

Under the experimental conditions, microbial activity was necessary for the formation of water-stable aggregates; and only after such aggregates were formed could roots synthesize larger structures by binding them together. These observations suggest that loss of structure may be due to destruction of conditions favoring growth of microorganisms. This is further suggested by the marked increase in structure formation that resulted from the addition of nutrient medium to the soil of cultures of molds and actinomycetes. Under irrigation, such conditions may arise from overwatering, depletion of organic matter, exhaustion of a necessary nutrient, or a detrimental increase in the salt content of the soil. In any case, remedial measures probably lie in the restoration of conditions favorable to the soil flora.

#### SUMMARY

The effect of bacteria, actinomycetes, and fungi and of roots of sudan grass on the formation of water-stable structures in two calcareous soils, Gila clay adobe and Tucumcari sandy loam, was studied by the wet-sieve method. The following results were observed:

Each group of microbes used formed a distinct type of aggregate.

The by-products of these organisms grown in liquid culture formed no aggregates in sterile soil. When water-stable structures were observed in these or any of the other treatments, living organisms were always observed in the structure.

Sudan grass grew weakly or not at all in sterile cultures, and the roots produced no soil structures.

When grown in soil cultures inoculated with a mixture of the microbial groups or contaminated by any one of them, sudan grass produced compound structures comprising microbial structures held together by roots.

Total aggregation was not affected by the presence of roots, but the structures were larger when roots were present.

#### REFERENCES

- (1) BAVER, L. D. 1940 *Soil Physics*. John Wiley & Son, Inc., New York.
- (2) FRED, E. B., AND WAKSMAN, S. A. 1928 *Laboratory Manual of General Microbiology* —with Special Reference to the Microorganisms of the Soil. McGraw-Hill Book Book Co., Inc., New York.
- (3) JOHNSON, J. 1919 The influence of heated soils on seed germination and plant growth. *Soil Sci.* 7: 1-103.
- (4) McCALLA, T. M. 1942 Influence of biological products on soil structure and infiltration. *Soil Sci. Soc. Amer. Proc.* 7: 209-214.
- (5) McCALLA, T. M. 1944 Influence of microorganisms and some organic substances on soil structure. *Soil Sci.* 59: 287-297.
- (6) McHENRY, J. R., AND RUSSELL, M. B. 1944 Microbial activity and aggregation of mixture of bentonite and sand. *Soil Sci.* 57: 351-357.
- (7) MARTIN, J. P., AND WAKSMAN, S. A. 1940 Influence of microorganisms on soil aggregation and soil erosion. *Soil Sci.* 50: 29-47.
- (8) MARTIN, J. P., AND WAKSMAN, S. A. 1941 Influence of microorganisms on soil aggregation and soil erosion: II. *Soil Sci.* 52: 381-394.
- (9) MARTIN, J. P. 1945 Microorganisms and soil aggregation: I. Origin and nature of some of the aggregating substances. *Soil Sci.* 59: 163-174.
- (10) MARTIN, T. L., AND ANDERSON, D. A. 1942 Organic matter decomposition, mold flora, and soil aggregation relationships. *Soil Sci. Soc. Amer. Proc.* 7: 215-217.
- (11) MYERS, H. E., AND McCALLA, T. M. 1941 Changes in soil aggregation in relation to bacterial numbers, hydrogen-ion concentration, and length of time soil was kept moist. *Soil Sci.* 51: 189-200.
- (12) PEELE, T. C. 1940 Microbial activity in relation to soil aggregation. *Jour. Amer. Soc. Agron.* 32: 664-668.
- (13) PEELE, T. C., AND BEALE, O. W. 1940 Influence of microbial activity upon aggregation and erodibility of lateritic soils. *Soil Soc. Amer. Proc.* 5: 33-35.
- (14) WAKSMAN, S. A. 1916 Soil fungi and their activities. *Soil Sci.* 11: 103-155.
- (15) WAKSMAN, S. A., AND MARTIN, J. P. 1939 The role of microorganisms in the conservation of the soil. *Science* 90: 304-305.



# PRODUCTIVITY AND ORGANIC MATTER LEVELS OF PALOUSE SILT LOAM AS AFFECTED BY ORGANIC RESIDUES AND NITROGEN FERTILIZERS<sup>1</sup>

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Increased susceptibility of the wheat lands of eastern Washington to erosion, coincident with the rapid loss of soil carbon and nitrogen reported elsewhere (2, 3, 4), prompted studies concerned with the maintenance of soil organic matter and productivity. The effects of repeated applications of organic residues and nitrogen fertilizers on wheat yields and on the carbon-nitrogen relationships in Palouse silt loam were first reported in 1938 by Holtz and Vandecaveye (1). It is the purpose of this paper to consider some of the changes which occurred in the surface foot of this soil during the 23-year period, 1921 to 1944, and to summarize the yield data for the same period.

Approximately 60 per cent of the precipitation received by this soil comes as winter snow or rain, from November through March, when temperature and evaporation are low. July and August are normally dry, with about 0.5 inch of rain each month. Wide extremes in total annual precipitation are common.

## EXPERIMENTAL PROCEDURE

The plots established in 1921 comprise an area known as field 3 on the College Farm. After being brought under cultivation between 1880 and 1891, the tract was used for a variety of crops until plots were established. Treatments as well as the cropping system are shown in table 1. With a few exceptions, yields have been determined by harvesting entire plots, and the binder stubble has been returned. Both alleys and roadways have been cropped similarly to adjacent 0.0372-acre plots.

Soil samples composited from 12 cores per plot were taken in 1921-22, 1934, and 1944, and were stored in closed glass jars. The surface samples taken in 1921-22 and in 1934 were analyzed in 1935-36; samples taken in 1944 and those obtained from the second 6 inches in 1922 were analyzed in 1944-45. Nitrogen was determined by a modified Gunning method, and carbon by a wet combustion procedure.

<sup>1</sup> Published as Scientific Paper No. 662, College of Agriculture and Agricultural Experiment Stations, State College of Washington, Pullman, Washington.

<sup>2</sup> Instructor and professor of soils, respectively. The field plots were under the direction H. F. Holtz, late associate in soils, prior to 1931, and of the junior author since that time. Aid in obtaining the field data was furnished by L. T. Kardos, formerly assistant professor of soils, L. C. Wheeting, research professor of soils, and G. O. Baker and G. M. Horner, formerly instructors in soils. J. H. Sackett, a former graduate assistant, made the chemical analyses for the 0- to 6-inch soil samples collected in 1921 and in 1934.

## EXPERIMENTAL RESULTS

*Crop yields*

Total and average yields for the period 1922-1944 are listed in table 1. Where wheat was grown annually, all plots that received large amounts of nitrogen fall into a high-yielding bracket. The average yield of the manured plot was lowered slightly by relatively frequent winter injury. From the plots cropped to wheat after fallow, yields were not greatly affected by treatment. Yields were in or near the range of 40 to 45 bushels per acre with the exception of 37.8 bushels for plot 203, to which straw was applied. The significance of this low value is questionable, since plot 303, treated identically but cropped in alternate years, had an average yield of 45.7 bushels per acre.<sup>3</sup>

*Nitrogen changes*

Data concerning nitrogen in the plots of field 3 are contained in table 2, as are certain calculations for the 23-year period. In the plots of the 100 series, where wheat was grown annually, little change occurred in the nitrogen level of the surface 6 inches of plots treated with NaNO<sub>3</sub> plus straw or with alfalfa hay plus straw. A slight increase in the nitrogen level resulted from applications of alfalfa hay or of straw plus (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and a considerable increase was effected by the use of manure. Losses of nitrogen resulted where no treatment was employed and from the use of straw or NaNO<sub>3</sub> alone. In the second 6 inches the nitrogen level was higher in 1944 than it had been in 1921 for all plots of the 100 series save the one receiving annual applications of alfalfa hay.

In plots of the 200 and 300 series, where wheat production was alternated with fallow, nitrogen was lost from the surface 6 inches of all plots. The treatments, although not effective in determining the direction of the change, did affect the degree of loss. In both series, maximum losses of nitrogen from the surface 6 inches occurred in the check plot and in the one to which NaNO<sub>3</sub> alone was added. The major loss of nitrogen from the surface 6 inches of all plots of the 200 series took place during the first part of the 1921-1944 period. The changes in the second 6 inches of these plots were comparatively small except in the plot receiving NaNO<sub>3</sub> alone, where the loss was appreciable, and in the plot receiving manure, where a definite gain took place. Among plots of the 300 series all changes indicated for the 1934-44 period, save those in plots receiving alfalfa or alfalfa plus straw, were sufficiently large to be of significance. Changes in the second 6 inches of these plots were small, with the exception of losses that occurred in the plots receiving NaNO<sub>3</sub> alone, NaNO<sub>3</sub> plus straw, and alfalfa alone.

A comparison between pounds of nitrogen added by application of organic residues and fertilizers, and pounds removed as a constituent of the grain or straw (table 2) shows that in all plots of the 100 series except those which received straw alone or no treatment, additions of nitrogen exceeded removals. Among the plots of the 200 and 300 series removals exceeded additions without exception.

<sup>3</sup> Detailed yield data are presented in Washington Agricultural Experiment Station Bulletin 476, 1946.

TABLE 1  
*Plot treatment and yields of wheat*

PLOT NUMBER	TREATMENT, POUNDS PER ACRE*	WHEAT YIELDS, 1922-1944, BUSHELS PER ACRE		
		Nitrogen	Total†	Average
<i>Annual wheat cropping system</i>				
100	2,700 wheat straw + 370 NaNO <sub>3</sub>	67	679	29.5
101	2,700 alfalfa hay	60	630	27.4
102	1,350 wheat straw + 1,350 alfalfa hay	35	541	23.5
103	2,700 wheat straw	10	443	19.3
104	None	0	457	19.9
105	2,700 wheat straw + 370 NaNO <sub>3</sub>	67	705	30.6
106	370 NaNO <sub>3</sub>	57	681	29.6
107	2,700 wheat straw + 286 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	69	714	31.0
108	12,000 farm manure	50	647	28.1
<i>Wheat-fallow cropping system</i>				
200	2,700 wheat straw + 370 NaNO <sub>3</sub>	67	504	42.0
300			489	44.5
201	2,700 alfalfa hay	60	480	40.0
301			521	47.4
202	1,350 wheat straw + 1,350 alfalfa hay	35	475	39.6
302			501	45.6
203	2,700 wheat straw	10	454	37.8
303			503	45.7
204	None	0	488	40.7
304			521	47.3
205	2,700 wheat straw + 370 NaNO <sub>3</sub>	67	521	43.5
305			515	46.8
206	370 NaNO <sub>3</sub>	57	532	44.4
306			490	44.6
207	2,700 wheat straw + 286 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	69	519	43.3
307			508	46.2
208	12,000 farm manure	50	501	41.8
308			484	44.0

\* All treatments are made just prior to fall plowing (annual wheat system) or early summer plowing (wheat-fallow system) except the NaNO<sub>3</sub> on plots 100, 200, and 300. This NaNO<sub>3</sub> is applied in March of the crop year.

† In the few instances in which individual data are lacking, the appropriate average yield has been employed in order to make the total production figures comparable.

of all plots during the initial 13 years and of all plots save the manured one from 1934 to 1944. The losses for the 23 years are of numerical significance except for

TABLE 3

*Carbon content of soils and effect of treatments on the carbon balance, 1921 to 1944*

PLOT NUMBER	TREATMENT	CARBON IN SOIL, PER CENT				CARBON, POUNDS PER ACRE FOOT* (1921-1944)		
		0-6 inches		6-12 inches		Gain or loss in soil	Added by residues†	Actual loss‡
		1921†	1934	1944	1921			
100	Straw + NaNO <sub>3</sub>	....	1.87	1.88	....	1.20	....	21,700
101	Alfalfa	1.84	2.00	1.97	1.47	1.52	3,000	21,700
102	Alfalfa + straw	1.88	1.96	1.94	1.33	1.47	3,500	21,700
103	Straw	1.94	1.91	1.98	1.52	1.59	1,900	21,700
104	None	1.90	1.80	1.78	1.39	1.54	800	0
105	Straw + NaNO <sub>3</sub>	1.90	1.99	1.97	1.38	1.63	5,700	21,700
106	NaNO <sub>3</sub>	2.00	1.98	1.92	1.43	1.61	2,000	0
107	Straw + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.94	2.15	2.23	1.46	1.85	11,900	21,700
108	Manure	1.98	2.42	2.45	1.63	1.93	13,200	30,600
200	Straw + NaNO <sub>3</sub>	....	1.69	1.68	....	1.32	....	11,300
201	Alfalfa	1.93	1.74	1.71	1.38	1.38	-3,600	11,300
202	Alfalfa + straw	1.95	1.73	1.71	1.33	1.42	-2,300	11,300
203	Straw	1.94	1.78	1.74	1.44	1.45	-3,100	11,300
204	None	1.87	1.74	1.61	1.46	1.44	-4,600	0
205	Straw + NaNO <sub>3</sub>	1.92	1.78	1.73	1.47	1.51	-2,400	11,300
206	NaNO <sub>3</sub>	1.95	1.78	1.70	1.44	1.40	-4,800	0
207	Straw + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.91	1.83	1.75	1.39	1.46	-1,400	11,300
208	Manure	1.95	1.88	1.91	1.39	1.60	3,200	16,000
300	Straw + NaNO <sub>3</sub>	....	1.85	1.73	....	1.44	....	10,400
301	Alfalfa	....	1.85	1.74	1.42	1.41	....	10,400
302	Alfalfa + straw	1.96	1.83	1.72	1.32	1.43	-1,900	10,400
303	Straw	1.91	1.81	1.71	1.38	1.32	-4,400	10,400
304	None	1.92	1.75	1.63	....	1.27	....	0
305	Straw + NaNO <sub>3</sub>	1.89	1.78	1.68	1.31	1.29	-3,800	10,400
306	NaNO <sub>3</sub>	1.87	1.69	1.64	1.32	1.29	-4,300	0
307	Straw + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.84	1.78	1.72	1.27	1.24	-2,500	10,400
308	Manure	1.86	1.92	1.84	1.53	1.53	-300	14,700
								15,000

\* Cores were taken in the field to determine the weight of dry soil per acre: 1,640,000 and 1,820,000 pounds in the first and second 6-inch sections, respectively.

† Some of the samples taken in 1921 were lost.

‡ Calculated from an average of several determinations of carbon contents of the straw, alfalfa hay, and manure used in two different years. Additions from stubble are not included.

§ The figures for actual loss represent the difference between the amounts of carbon in the soil in 1944, and the sum of the carbon in the soil in 1921 and the amounts supplied by means of the organic materials.

the manured plot. Changes in the second 6 inches during the 23-year experimental period are too small to be of much significance, if the gain recorded for

plot 208 is excepted. Data for carbon changes, expressed as pounds per acre foot, are all negative except the value for the manured plot.

For plots of the 300 series, plot 208 excepted, the data are in general agreement with those for companion plots of the 200 group. Plots 208 and 308 are in agreement in that slight changes with respect to carbon occurred in the surface 6 inches, but in plot 308 no change was found in the 6- to 12-inch section, whereas a marked gain in carbon was found at that depth in plot 208. When carbon changes are expressed as pounds per acre foot of soil, all values are negative for plots of the 300 series.

In the last column of table 3 are listed actual losses of carbon, considering additions in the form of organic materials applied and gains or losses in the first foot of soil. Where wheat was grown annually (100 series), carbon losses occurred except where no materials other than binder stubble were returned and where  $\text{NaNO}_3$  alone was applied. The figures for these two plots are small, but their significance is supported by the fact that the equivalent plots under a wheat-fallow farming system suffered minimum losses of carbon. In view of the quantities of organic residues added, the actual losses of carbon in all plots of the 200 and 300 series were relatively larger than those in the corresponding plots of the 100 series.

#### *Carbon-nitrogen ratios*

Slight increases, at the close of the initial 13-year period, in the carbon-nitrogen ratios of the surface 6 inches of all plots save 307 have been mentioned previously (1). Inability of any of the treatments either to widen or to narrow these ratios to a significant degree was also pointed out. During the 1934-1944 period changes in the carbon-nitrogen ratios continued to be slight and failed even to show the general positive trend that was evident during the initial 13-year period. The average ratio for the surface 6 inches of all plots in 1944 was 11.65 as compared with 11.78 in 1934 and with 11.20 in 1921.

Changes in the carbon-nitrogen ratios of the second 6 inches of soil, during the 1921-1944 period, were also slight but rather consistent as to direction. The values were in general somewhat below those for the first 6 inches, a phenomenon which has been found to occur in many other soils.

#### *Application to farming practices*

The practice of burning crop residues was once general and is still common in the area where Palouse silt loam is the dominant soil type. Straw residues are burned in this area for two reasons: first, because of the difficulty encountered in the plowing, cultivating, and drilling operations when the residue is heavy; and second, because of the possible yield depression resulting from lack of available nitrogen for the following cereal crop if the residue is plowed under.

Plowing under 2,700 pounds of straw per acre prior to the fallow period did not reduce the supply of available nitrogen sufficiently to lower the yield to a significant degree in the experimental plots. Plowing under a high-nitrogen residue or inorganic nitrogen fertilizers was equally ineffective in increasing the yield.

In the area under consideration moisture is seldom so limited that a fallow period is necessary to store water. In recent years annual cropping involving wheat and peas, principally, has been widely practiced. Weed control has been one of the principal field problems aggravated by this crop sequence. If suitable weed control were effected, such a system would have merit with respect to erosion control and to organic matter maintenance. Data for the plots which are cropped annually to wheat indicate that if supplemental nitrogen is used with highly carbonaceous residues, not only is the yield increased to a marked degree, but in addition the soil organic matter level is either maintained or improved. The plowing under of binder stubble plus 2,700 pounds of straw each year, as compared with plowing under binder stubble alone, has resulted neither in the conservation of appreciable quantities of soil nitrogen and organic matter nor in a significant decrease in yield.<sup>4</sup>

#### SUMMARY AND CONCLUSIONS

Three groups of plots were used during the 23-year period from 1921 to 1944 to investigate the effect of organic residues and nitrogen fertilizers on Palouse silt loam soil under two cropping systems: continuous wheat, and wheat after fallow.

Under an annual wheat-farming system, nitrogen was the principal factor which determined crop yields. Those plots which received annual applications of nitrogen in amounts ranging from 50 pounds to 69 pounds per acre produced an average yield of 29.4 bushels per acre, as compared with 19.9 bushels from the untreated plot. Applications of straw without supplemental nitrogen resulted in an average yield of 19.3 bushels per acre.

Yields from all plots which received heavy biennial applications of nitrogen in some form and which were cropped and fallowed in alternate years averaged 44.1 bushels per acre as compared with 44.0 bushels from the two untreated plots. Where straw was employed without supplemental nitrogen the average yield was 41.8 bushels.

During the 23-year experimental period the nitrogen content of the surface 6 inches of plots which were cropped annually to wheat was decreased as a result of no treatment, of the application of  $\text{NaNO}_3$ , alone, or of straw alone. It was not affected appreciably by annual applications of  $\text{NaNO}_3$  plus straw, or alfalfa hay plus straw, but was increased slightly by annual applications of alfalfa hay, or straw plus  $(\text{NH}_4)_2\text{SO}_4$  and considerably by the use of manure. In the second 6 inches, the nitrogen content was increased in all plots except the one receiving alfalfa hay. Within the surface foot of soil small losses of nitrogen were suffered by the untreated plot and the one receiving straw only. Essentially no change resulted from the application of  $\text{NaNO}_3$  alone, and an increased nitrogen content was realized from the other treatments.

<sup>4</sup> An important point, unanswered by these data, would be the effect of larger quantities of straw on the yield and on soil changes. Total straw produced from the wheat-fallow plots has approached 6,000 pounds per acre in favorable years. The corresponding figure is approximately 3,500 pounds for the fertilized plots which are cropped every year.

In plots on which wheat production was alternated with fallow, nitrogen was lost from the surface 6 inches of the soil of all plots. Maximum losses occurred in the check plots and in those to which  $\text{NaNO}_3$  alone was added. There was a loss of nitrogen from the surface foot of soil of all plots.

Small increases in carbon content in the surface 6 inches of plots cropped annually to wheat resulted from the application of  $\text{NaNO}_3$  alone and from no treatment, whereas gains were realized following the application of manure, or straw plus  $(\text{NH}_4)_2\text{SO}_4$ . In the second 6 inches the carbon content was increased for all plots during the 23-year period. In the surface foot as a whole, gains in carbon were obtained in all plots. These gains were small, however, in the untreated plot and in those receiving straw alone or  $\text{NaNO}_3$  alone. Among plots that were fallowed and cropped in alternate years, carbon was lost from the surface foot as well as from the surface 6 inches, except in the manured plots.

During the 1934-1944 period changes in the carbon-nitrogen ratios of the surface 6 inches of all plots were slight and failed to show the general positive trend of the first 13 years. Changes in the carbon-nitrogen ratios of the second 6 inches of soil during the 23-year period were slight but consistently positive.

The results of the 23-year experiment point to the conclusion that the wheat-fallow system of cropping depletes soil fertility. Soil nitrogen and organic matter continued to decline regardless of biennial applications of nitrogen fertilizers and crop residues alone or combined.

An annual cropping system in which crop residues are utilized and supplemented with nitrogen fertilizers is more promising in conserving soil fertility. Yields were well above those from untreated plots, and the supply of soil organic matter was maintained or improved.

#### REFERENCES

- (1) HOLTZ, H. F., AND VANDECAYEYE, S. C. 1938 Organic residues and nitrogen fertilizers in relation to the productivity and humus content of Palouse silt loam. *Soil Sci.* 45: 143-163.
- (2) HORNER, G. M., MCCALL, A. G., AND BELL, F. G. 1944 Investigations in erosion control and the reclamation of eroded land at the Palouse Conservation Experiment Station, Pullman, Washington, 1931-1942 U. S. Dept. Agr. Tech. Bul. 860.
- (3) SIEVERS, F. J., AND HOLTZ, H. F. 1922 The silt loam soils of eastern Washington and their management. Wash. Agr. Exp. Sta. Bul. 166.
- (4) THATCHER, R. W. 1912 The nitrogen and humus problem in dry farming. Wash. Agr. Exp. Sta. Bul. 105.



# PHOSPHATE FIXATION IN ACID SOILS AND ITS MECHANISM

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Despite a great amount of research by many workers over the years, the actual process whereby phosphate fixation takes place in soils is still obscure.

Some recent contributors hold the view that, within certain ranges of reaction at least, phosphorus is adsorptively fixed on the surface of clay particles (1, 11, 13, 14, 15, 17, 18). Among the several theories of this adsorptive fixation are those of adsorption through one or more free aluminum valences, displacement of OH groups from an aluminosilicate structure, and replacement of 2 Si by a P-Al complex.

On the other hand, several authors favor the chemical theory. According to them, the phosphorus-fixing capacity of most acid soils under field conditions can be very largely accounted for by the precipitation of the soluble phosphorus as iron and aluminum phosphates, and fixation at particle surfaces in a replaceable form is thought to be of slight practical significance. This conclusion is based mainly on the observed reduction in the phosphorus-fixing capacity of soils by the removal of the free sesquioxides (2) and also on the correlation of the fixing capacity with the free sesquioxides of soils (12). Ghani (7) found that the fixing power of acid soils was considerably reduced by chemical deactivation of the iron and aluminum by means of 8-hydroxyquinoline, and the results were taken to indicate that fixation in acid soils was largely due to the formation of iron and aluminum phosphates by chemical interaction between the soluble phosphorus and hydrated oxides of iron and aluminum. Ghani and Aleem (6), in a study of the distribution of different forms of phosphorus in a number of Indian soils, found that iron and aluminum phosphates were relatively high in acid soils and concluded that under acid conditions accumulation of phosphorus takes place mainly in these forms.

In the work reported here more direct evidence has been adduced in favor of the chemical precipitation theory of fixation, by actual determination of the iron and aluminum phosphates formed after addition of soluble phosphorus. The chemical transformation of the added phosphorus in the soil-phosphate mixture has been studied by the fractionation method of Dean (3) modified by Ghani (5). A complete fractionation of the treated soil samples at the beginning of the experiment and at periodical intervals thereafter will not only show the rate and amount of fixation but will also reveal more definitely the nature of phosphates formed in soils through fixation of applied soluble phosphorus. Though fixation studies in the ordinary way are found to be numerous in the relevant literature, few attempts are known to have been made in the past to account for the fixed phosphorus in a straightforward way. The present line of investigation represents a departure from the methods hitherto followed in such studies. In

In this connection the work of Heck (9) may be mentioned. He made a preliminary study of the nature of phosphates formed in soils through fixation by comparing the solubility curves of different phosphates of known chemical nature with those of the phosphates in soils before and after treatment with soluble phosphate. Applying this method to four soils of different origin, he found that in one of the soils (Miami silt loam) the soluble phosphate was largely fixed as calcium phosphate. In another (Carrington silt loam), which was more acid, less calcium phosphate and more iron and aluminum phosphates were formed. In the two other soils (Hawaiian laterites), fixation was largely in the form of basic iron and aluminum phosphates.

As the phenomenon of fixation appears in an acute form in the acid soils of India, the investigation reported here was confined to acid soils. The work is divided into two parts. In part 1 fixation experiments have been carried out with mixtures of soils and phosphatic materials incubated under conditions approximating those in the field. The time of contact varied from immediate to 6 weeks. In part 2, fixation has been studied under purely laboratory conditions, mixing of the soil and the phosphate solution having been done by mechanical shaking for known periods. The time of contact varied from immediate to 24 hours.

#### PART 1. FIXATION STUDY ON INCUBATED SAMPLES

##### *Soil and phosphatic materials used*

Two soils were selected for this study, one from an unmanured fallow plot of the Bengal Central Farm at Dacca and the other from an unmanured experimental plot of the Berhampur Farm. The pH values of the soils were 5.2 and 4.6 respectively. In the latter soil, single and double doses of superphosphate applied from year to year made no change in the available phosphorus content. As the aim of any laboratory method is to develop a standardized procedure which will throw adequate light on the practical problems of agriculture, phosphatic fertilizers such as Nicifos and bonemeal were used as phosphorus-supplying materials instead of the chemically pure salts of phosphoric acid. These fertilizers are generally used in this country for supplying nitrogen and phosphorus to the soil. Nicifos 2/1 contains 12 and 19 per cent of  $P_2O_5$  and N, respectively; Nicifos 4 contains 58 and 12.2 per cent of  $P_2O_5$  and N, respectively; and bonemeal contains 22 and 3.5 per cent of  $P_2O_5$  and N, respectively. Because of war conditions, superphosphate could not be procured.

##### *Experimental procedure*

Samples weighing 0.1 and 0.2 gm. of each of the three fertilizers were thoroughly mixed with 150-gm. samples of the soil—the equivalent of adding 8 and 16 mgm.  $P_2O_5$  from Nicifos 2/1 per 100 gm. of soil, 38.6 and 77.2 mgm.  $P_2O_5$  from Nicifos 4, and 14.6 and 29.2 mgm.  $P_2O_5$  from bonemeal. Of each of these mixtures, 50 gm. was weighed into a separate wide-mouthed flask, so that the experiments were performed in triplicate. To each mixture enough distilled water was added to bring it to its optimum moisture content. The flasks were then

weighed, stopped with cotton plugs, and stored in a dark cupboard. Every few days the flasks were aerated and reweighed, and water was added to compensate for the loss due to evaporation. At intervals of 1, 2, 4, and 6 weeks following the incubation, samples were withdrawn from the incubating flasks, air-dried, and analyzed for phosphorus fractions. Samples were also analyzed after mixing the fertilizers without incubation. The treatment of the control was exactly the same except that no fertilizer was added to it.

*Results of fixation study on incubated samples of Berhampur soil*

The effects of time of contact and amount and kind of fertilizer on the main inorganic phosphorus fractions in the soil-fertilizer mixtures are shown in tables 1 and 2.

TABLE 1

*Change in available phosphorus in Berhampur soil with time, amount and kind of fertilizer*

Mgm. P<sub>2</sub>O<sub>5</sub> per 100 gm. of soil

FERTILIZER		IMMEDIATE	1ST WEEK	2ND WEEK	4TH WEEK	6TH WEEK
Kind	Amount					
Nicifos 2/1	8.0	3.1	2.8	3.2	3.0	2.6
	16.0	9.0	4.0	3.7	3.8	3.6
Nicifos 4	38.6	18.0	12.8	10.5	9.0	9.0
	77.2	45.0	32.0	25.0	24.5	25.0
Bonemeal	14.6	8.2	4.7	4.8	4.5	4.5
	29.2	18.5	14.0	11.2	9.0	9.0
Control	....	1.8	1.8	1.6	1.6	1.8

It will be seen from table 1 that immediately after mixing of the soil with the fertilizer, a large amount of the added phosphorus was fixed, inasmuch as it could not be wholly recovered in the acetic acid extract. The amount fixed varied with the quantity of phosphorus added to the soil and also according to the nature of the fertilizer supplying the phosphorus.

As the amount of phosphorus added increased, the amount of available phosphorus also increased, though not in the same proportion. As the time of contact between the phosphorus compound and the soil increased, the available phosphorus tended to decrease, then remained nearly constant after 2 weeks in all cases. The amount of phosphorus added exerted a great effect on the rate of solubility. Thus, in the case of Nicifos 2/1, single dose the lowest dose of the lot—the availability was the same from the time of mixing onward, that is, maximum fixation took place immediately after mixing. As the amount increased, as in Nicifos 2/1, double dose, and in bonemeal, single dose, availability fell during the first week and then remained constant. As the dose still

further increased, equilibrium was not reached until after the second week of incubation.

From table 2 it will be seen that the addition of fertilizers increased the iron and aluminum phosphate fraction of the soil. Here also, as the amount of added phosphorus increased the amount of iron and aluminum phosphate increased, though not in the same proportion. With high doses the amount remained virtually constant until the 4th week, after which there was a slight increase. With the lower doses the amount tended to decrease somewhat from the start and then slightly increased after the 4th week. Obviously the fluctuation was due to the fact that the conditions leading to an equilibrium were rapidly changing during the time of experiments.

TABLE 2

*Change in iron and aluminum phosphates and apatite phosphate in Berhampur soil with time, amount and kind of fertilizers*

Mgm. P<sub>2</sub>O<sub>5</sub> per 100 gm. of soil

FERTILIZER		IMMEDIATE		1ST WEEK		2ND WEEK		4TH WEEK		6TH WEEK	
Kind	Amount	Fe & Al PO <sub>4</sub>	Apatite PO <sub>4</sub>								
Nicifos 2/1	8	43	28	43	23	38	26	34	27	39	35
	16	53	30	43	23	38	28	37	30	42	31
Nicifos 4	38.6	56	30	56	24	56	28	53	32	65	40
	77.2	72	31	70	22	67	26	67	32	77	37
Bonemeal	14.6	45	31	35	21	35	22	40	40	40	34
	29.2	50	31	45	21	44	25	42	35	50	34
Control		35	25	35	22	26	23	27	30	30	30

Table 2 shows the change in the apatite phosphate with time. Contrary to the findings for iron and aluminum phosphates, this fraction underwent comparatively little change due to the application of the fertilizer and due to incubation. In almost all the treatments this fraction decreased during the first week but again rose above the control value with time. The final increase over the control at the end of 6 weeks was, however, comparatively small, showing that the soluble phosphorus did not revert to the apatite form to any very great extent. The disappearance of the easily soluble phosphorus appears, thus, to be due mainly to an increase in the iron and aluminum phosphate fraction of the soil.

The actual amounts of P<sub>2</sub>O<sub>5</sub> fixed in the various treatments are shown in table 3. These figures were obtained by subtracting the available P<sub>2</sub>O<sub>5</sub> of the treated samples from the sum of the P<sub>2</sub>O<sub>5</sub> added and the available P<sub>2</sub>O<sub>5</sub> of the control sample. As the untreated soil always contains some available phosphorus which comes into solution under the conditions of extraction adopted, this control

value should be subtracted from the value obtained so as to get the portion which has come from the added phosphorus.

From table 3 it is evident that as the amount of phosphorus added increased, the amount fixed also increased, though not in the same proportion. As the time of incubation increased, the rate of fixation also increased but after a time remained virtually constant. It is seen from table 1 that the available phosphorus increased with the amount of added phosphorus, whereas table 3 shows that fixed phosphorus also increased with increase in the amount of phosphorus added. As the two quantities may vary differently, the true significance of variation of fixation with time and dose will be revealed when the fixed phosphorus is expressed as percentage of the added  $P_2O_5$ . In table 3 the results are also expressed on the percentage basis, from which it is evident that the percentage of  $P_2O_5$  fixed at any time was lower at the higher dose. Of the four doses of Nicifos used, the fixation at the lower doses was always at a higher level than

TABLE 3  
*Phosphate fixed in Berhampur soil under various treatments*  
Mgm.  $P_2O_5$  per 100 gm. of soil

FERTILIZER		IMMEDIATE		1ST WEEK		2ND WEEK		4TH WEEK		6TH WEEK	
Kind	Amount	Amt. fixed	Per cent fixed								
Nicifos 2/1	8	7	84	7	88	6	80	7	83	7	90
	16	9	55	14	86	14	87	14	86	14	89
Nicifos 4	38.6	22	58	28	72	30	77	31	81	31	81
	77.2	34	44	47	61	54	70	54	70	54	70
Bonemeal	14.6	8	56	12	80	11	78	12	80	12	82
	29.2	13	43	17	58	20	67	22	75	22	75

that at higher doses. In the case of bonemeal, double dose, the concentration of which is less than that of Nicifos 4, single dose, the fixation was at a much lower level than that of the latter. This shows that for similar doses the phosphorus of bonemeal was less readily fixed than the phosphorus of Nicifos. When the effect of time on the degree of fixation is considered, it will be observed that, at all doses, percentage fixation rose very rapidly during the first one or two weeks, after which it changed slowly and remained nearly constant. It will be further noticed that 43 to 84 per cent of the added phosphorus was fixed at the start of incubation. As the contact period increased to 6 weeks, these figures rose to 70 and 90 per cent respectively.

Many investigators have shown that phosphate fixation is a time reaction and may continue for months. Heck (9) made a fixation study of four soils at intervals of 2, 10, 40, and 80 days and showed that most pronounced fixation took place within a few hours, and that after a transition period, varying from 2 to 10 days, the fixation took the form of a virtually straight line with a tendency to

be somewhat asymptotic. Scarseth and Tidmore (17) found that when very soluble phosphates were added to an acid soil the fixation of  $\text{PO}_4$  ion took place as rapidly as the phosphate was diffused throughout the soil and came in contact with the colloids. According to Hibbard (10) most of the added  $\text{PO}_4$  is fixed within a few hours and that fixation may continue for a year or more. The decrease in the rate of fixation with time has been ascribed to the diminution of the avidity of the soil for  $\text{PO}_4$  and to the lowering of the concentration of the uncombined  $\text{P}_2\text{O}_5$  remaining in the solution. The results obtained by Hibbard (10) and Gile (8) reveal that the larger the amount of  $\text{P}_2\text{O}_5$  added, the less firmly it is held by the soil against the solvent action of the extracting reagent.

An attempt was next made to correlate the fixed phosphorus with the phosphorus recovered as iron and aluminum phosphate in the alkaline extraction and to find out the degree to which the chemical precipitation is responsible for the

TABLE 4

*Phosphorus recovered from Berhampur soil as iron and aluminum phosphates*

Amount obtained from treated sample — amount obtained from control sample.  
Mgm.  $\text{P}_2\text{O}_5$  per 100 gm. soil

FERTILIZER		IMMEDIATE	1ST WEEK	2ND WEEK	4TH WEEK	6TH WEEK
Kind	Amount					
Nicifos 2/1	8	8	8	12	7	9
	16	18	8	12	10	12
Nicifos 4	38.6	21	21	30	26	35
	77.2	37	35	40	40	47
Bonemeal	14.6	10	5	9	13	10
	29.2	15	10	18	15	20

phenomenon of fixation. In table 4 are shown the increases in the iron and aluminum phosphate fraction of the soil due to the various treatments under consideration. Each figure was obtained by subtracting the alkali-soluble inorganic phosphorus of the control sample from that of the treated sample. The results reveal that as the added  $\text{P}_2\text{O}_5$  increased, more and more iron and aluminum phosphate was formed. Moreover, as the time of contact advanced, this reaction took place to a greater extent.

The fixation and recovery values are compared in table 5, from which it appears that in the majority of cases the amount of  $\text{P}_2\text{O}_5$  recovered nearly approached the amount fixed. If the relation between these two quantities were shown in a scatter diagram, the points would be found to group along the line of theoretical or full recovery. The majority of the points would fall below the line, showing that recovery was not 100 per cent. A few points would be found to lie a little above the line, from which it may be taken that the incomplete recovery was due to experimental errors, in which case the formation of iron and aluminum phosphate would appear to be solely responsible for the process of

fixation, at least in the soil under investigation. Even if an experimental line were drawn covering as many of the plotted points as possible, its deviation from the theoretical line would show that at least 90 per cent of the fixation can be accounted for by chemical precipitation. The findings recorded here are important not only because such a treatment of the results will enable one to investigate the nature and extent of chemical fixation in a particular soil but also because, within the knowledge of the investigators, more direct and definite evidence of the chemical theory of fixation has not been offered elsewhere.

#### *Fixation study with Dacca soil*

The Dacca soil is much less acid than the Berhampur soil, its pH being 5.2, and its sesquioxide content is also much lower than that of the Berhampur soil.

TABLE 5

*Relation between amount of  $P_2O_5$  fixed and amount recovered as iron and aluminum phosphates in Berhampur soil*

Mgm.  $P_2O_5$  per 100 gm. of soil

FERTILIZER		IMMEDIATE		1ST WEEK		2ND WEEK		4TH WEEK		6TH WEEK	
Kind	Amount	Fixed	Recover-	Fixed	Recover-	Fixed	Recover-	Fixed	Recover-	Fixed	Recover-
Nicifos 2/1	8	7	8	7	8	6	12	7	7	7	9
	16	9	18	14	7	14	12	14	10	14	12
Nicifos 4	38.6	22	21	28	21	30	30	31	26	21	35
	77.2	34	37	47	35	54	40	54	40	54	47
Bonemeal	14.6	8	10	12	5	11	9	12	13	12	10
	29.2	13	15	17	10	20	18	22	15	22	20

The Dacca soil contains much more available phosphorus—18 mgm.  $P_2O_5$  per 100 gm. The incubation experiments and the fixation study were made in exactly the same manner as in the Berhampur soil except that the incubation experiments with the Dacca soil were concluded at the end of the fourth week. The same fertilizers were used and the amounts applied were also the same.

The changes in the quantity of available phosphorus, fixed phosphorus, and percentage fixation are shown in tables 6 and 7. The tables show that in this soil also the major part of the fixation took place within a very short time of the mixing of the fertilizers with the soil, though the actual amount of fixation was comparatively small. As before, with the increase in the amount of  $P_2O_5$  added, both the available phosphorus and the fixed phosphorus increased, but the rate of increase was much less than was found in Berhampur soil. By increasing the dose from 8 mgm. to 77 mgm.  $P_2O_5$  in the form of Nicifos, the fixed phosphorus in Berhampur soil was increased from 7 mgm. to 34 mgm. (table 3), whereas in this case it increased from 5 mgm. to 23 mgm. under identical conditions.

Doubling the dose of bonemeal produced a change of about 4 mgm. in the

phosphate fixed in the Berhampur soil, whereas in the Dacca soil no significant change was observed. Percentage fixation also was much less in the Dacca soil in various treatments, and it varied with the amount of  $P_2O_5$  added. When the effect of time on the magnitude of the changes produced was considered, the two soils were found to differ fundamentally. In the Berhampur soil, the available phosphorus at first decreased progressively with time, depending on the concentration of added  $P_2O_5$ , and finally remained virtually constant (table 1). In the Dacca soil, this quantity did not decrease appreciably with time except with the highest amount of Nicifos; the bonemeal-treated samples, on the other hand, showed an increase in the available phosphorus with time. In the Berhampur soil, percentage fixation rose very rapidly with time to a period of 4 weeks, after which it changed only very slowly. In the Dacca soil in most of the Nicifos treatments, an increase was shown in the first week and then the quantity fell.

TABLE 6

*Change in available phosphate in Dacca soil with time, amount, and kind of fertilizer*  
Mgm.  $P_2O_5$  per 100 gm. of soil

FERTILIZER		IMMEDIATE	1ST WEEK	2ND WEEK	4TH WEEK
Kind	Amount				
Nicifos 2/1	8.0	21	20	21	20
	16.0	24	23	25	28
Nicifos 4	38.6	44	39	40	42
	77.2	72	71	65	60
Bonemeal	14.6	24	27	27	35
	29.2	40	41	45	49
Control	—	18	18	18	18

A steady increase was maintained only in treatments receiving the largest quantity of Nicifos.

The decrease in percentage fixation with time is not easily understood, as it would imply that phosphorus once fixed becomes released later as time progresses. Such a probability, however, cannot be completely ruled out, as the phosphate fixation represents a rather slowly changing equilibrium in which the opposing reaction also has its part to play. Moreover, it has often been said by various investigators that the stability of the phosphorus fixed varies from soil to soil depending on the soil factors influencing phosphate fixation. It may be that during the first stages fixation represents the formation of moderately fixed (or moderately available) phosphorus, which with the progress of time gives way to the opposing forces, releasing a part of it in the available form. It may also be that, depending on the degree of hydration of the sesquioxides, which are the active agents in chemical fixation, an unstable loose combination of the  $PO_4$  ion with these materials is formed. The applied phosphate would thus be

temporarily withheld from the solution, instead of being permanently locked up in the soil. In view of the low fixing power of this soil and in view of its power of easy saturation, as has already been shown, such an eventuality is highly probable. Hibbard (10), in his study of time effect in fixation, found that in one out of six soils water-soluble phosphorus steadily increased to a great extent from 1 hour to 2 months, though a large percentage of the added phosphorus ( $\text{CaHPO}_4$ ) was fixed in 1 hour. The soil was sandy loam of pH 8.2 and contained a high amount of available phosphorus (Truog's). The author asserted that "no explanation of this variation is apparent."

The point in question will be made clearer by a reference to the behavior of bonemeal toward fixation. Table 7 shows that with bonemeal both the amount of  $\text{P}_2\text{O}_5$  fixed and the percentage fixation fell progressively with time, and after a period of 4 weeks virtually all of the phosphorus supplied had returned to the available form. It would appear that in this soil if bonemeal is applied suffi-

TABLE 7  
*Phosphate fixed in Dacca soil under various treatments*  
Mgm.  $\text{P}_2\text{O}_5$  per 100 gm. of soil

FERTILIZER		IMMEDIATE		1ST WEEK		2ND WEEK		4TH WEEK	
Kind	Amount	Amount fixed	Per cent fixed						
Nicifos 2/1	8	5	63	6	71	5	63	6	74
	16	10	67	11	68	9	59	6	40
Nicifos 4	38.6	13	33	18	46	17	43	15	38
	77.2	23	30	24	31	30	39	35	45
Bonemeal	14.6	9	59	6	43	6	41	-2	..
	29.2	7	25	6	23	2	8	-1	..

ciently early, none of its phosphorus will be wasted by fixation. The calcium that is present in the bonemeal might be instrumental in the process of reconversion. In this respect, bonemeal may be regarded as a superior fertilizer to Nicifos, at least for types of soil like the Dacca.

It has been seen that, in the Dacca soil, fixation took place in a much less degree than in the Berhampur in all the treatments. Evidently the increase, if any, in the iron and aluminum phosphate fraction and in the apatite phosphate should be correspondingly low. Table 8 shows the changes produced in these two fractions by the addition of the phosphate materials, and table 9 shows the amount of phosphorus recovered as iron and aluminum phosphates in the different treatments.

It will be seen from table 8 that apatite phosphate underwent no change due to the treatments, showing that none of the added phosphorus was reconverted to this form. In the iron and aluminum phosphate fraction, however, variable increases were effected, but they are, in all cases, very slight, as is evident from

table 9. Furthermore, with bonemeal, the treatments in which no phosphorus was fixed reveal little increase in the iron and aluminum phosphate content of the soil. As before, the fixation and recovery values are compared in table 10. The data show that in this soil, as well as in the Berhampur, a very high per-

TABLE 8

*Change in iron and aluminum phosphates and apatite phosphate in Dacca soil with time, amount, and kind of fertilizer*

Mgm. P<sub>2</sub>O<sub>5</sub> per 100 gm. of soil

FERTILIZER		IMMEDIATE		1ST WEEK		2ND WEEK		4TH WEEK	
Kind	Amount	Fe & Al PO <sub>4</sub>	Apatite PO <sub>4</sub>	Fe & Al PO <sub>4</sub>	Apatite PO <sub>4</sub>	Fe & Al PO <sub>4</sub>	Apatite PO <sub>4</sub>	Fe & Al PO <sub>4</sub>	Apatite PO <sub>4</sub>
Nicifos 2/1	8	84	40	85	41	80	38	86	38
	16	84	40	87	41	82	39	86	40
Nicifos 4	38.6	92	40	99	40	90	39	100	37
	77.2	100	40	105	40	106	40	100	39
Bonemeal	14.6	84	40	87	40	86	40	80	40
	29.2	84	40	84	40	85	40	78	40
Control	....	80	40	80	40	80	41	80	40

TABLE 9

*Phosphorus recovered from Dacca soil as iron and aluminum phosphates  
Amount obtained from treated sample — amount obtained from control sample.*

Mgm. P<sub>2</sub>O<sub>5</sub> per 100 gm. soil

FERTILIZER		IMMEDIATE	1ST WEEK	2ND WEEK	4TH WEEK
Kind	Amount				
Nicifos 2/1	8.0	4	5.7	nil	6
	16.0	4	7.3	2	6
Nicifos 4	38.6	12	18.6	10	20
	77.2	20	24.6	26	20
Bonemeal	14.6	4	6.6	6	nil
	29.2	4	4.0	5	nil

centage of the fixed phosphorus was recovered as iron and aluminum phosphates. The fixing power of the Dacca soil was lower than that of the Berhampur.

The foregoing fixation study substantiates the view that in acid soils fixation is mainly due to the formation of insoluble phosphates of iron and aluminum. Ford (4) and Heck (9) have shown that such a reaction depends not only on the amount of free sesquioxides present in the soil but also their state of hydration.

The reaction of the soil, again, is an important factor in governing the quantity of the oxides of iron and aluminum and their degree of hydration. The difference in the fixing power of the two soils studied here may be ascribed to the variation of these factors.

#### PART 2. FIXATION STUDY AT SHORT-TIME INTERVALS

Fixation was next studied under purely laboratory conditions, and the mixing of the soil and fertilizer was done by mechanical shaking for known periods.

TABLE 10

*Relation between  $P_2O_5$  fixed and amount recovered as iron and aluminum phosphates in*

*Dacca soil*

Mgm.  $P_2O_5$  per 100 gm. of soil

FERTILIZER		IMMEDIATE		1ST WEEK		2ND WEEK		4TH WEEK	
Kind	Amount	Fixed	Recovered	Fixed	Recovered	Fixed	Recovered	Fixed	Recovered
Nicifos 2/1	8	5.0	4.0	5.7	5.3	5.0	0.0	5.9	6
	16	10.5	4.0	10.7	7.3	9.4	2.0	6.4	6
Nicifos 4	38.6	12.6	12.0	17.6	18.6	16.6	10.0	14.6	20
	77.2	23.2	20.0	24.0	24.6	30.0	26.0	35.2	20
Bonemeal	14.6	8.6	4.0	6.1	6.6	6.0	6.0	nil	nil
	29.2	7.2	4.0	6.6	4.0	2.2	5.0	nil	nil

In addition to the Berhampur soil, unmanured acid soils from Kanke, Karimganj, and Titamari were included in this study. The pH values of these soils were 5.6, 4.5, and 4.7 respectively.

#### Experimental procedure

Samples of soil weighing 2.5 gm. each were mixed in different bottles with 250 cc. of phosphate solution of known  $P_2O_5$  content prepared by dissolving Nicifos 2/1 in 0.5*N* acetic acid. The mixtures were shaken in a mechanical shaker for 2, 6, and 24 hours, and filtered immediately through phosphorus-free paper. One of the bottles was shaken by hand for about half a minute immediately after mixing and then quickly filtered. The  $P_2O_5$  was determined colorimetrically in a suitable aliquot of the filtrate. The fixed  $P_2O_5$  was determined by subtracting the amount obtained in the filtrate from the amount added to the solution. Since the solvent had itself extracted some phosphate from the soil, the amount obtained in the filtrate did not give the amount actually fixed from the added phosphorus. Control experiments were, therefore, performed under completely identical conditions, except that no phosphate was added to the acetic acid. The phosphate recovered was the amount obtained from the filtrate less that obtained from the control. The fixed phosphorus was calculated by subtracting the recovered amount from that added. The residue in the filter

paper and that left in the bottle were shaken with 250 cc. of 0.25*N* NaOH for 2 hours, and the inorganic  $P_2O_5$  was determined in a suitable aliquot of the filtered extract. The increase in the alkali-soluble phosphate in the treated soil over that obtained from the control experiment was due to fixation and gave a measure of the recovery of the fixed phosphorus as iron and aluminum phosphate.

### Results

The results, presented in table 11, show that fixation took place immediately on mixing the soil with fertilizer and that it regularly increased with time through the 24-hour contact period, though up to a period of 6 hours the fixation is very rapid. It also shows that with Nicifos as the source of phosphorus, the rate of fixation does not attain the maximum in such a short time as 30 minutes, or 1 hour, as has been found by some of the previous workers (16). It has already been shown that in the Dacca and Berhampur soils (incubated samples under conditions approximating those in the field) the  $P_2O_5$  recovered

TABLE 11  
*Relation between amount of  $P_2O_5$  fixed by soils and that recovered as iron and aluminum phosphates*

Mgm.  $P_2O_5$  per 100 gm. of soil

TIME	BERHAMPUR		KANKE		KARIMGANJ		TITAMARI	
	Fixed	Recovered	Fixed	Recovered	Fixed	Recovered	Fixed	Recovered
Immediate.....	4.2	6.5	0.2	1.8	2.0	2.5	1.4	0.8
2 hours.....	14.2	16.1	7.0	6.5	6.5	7.4	3.4	4.5
6 hours.....	16.5	16.9	9.8	8.9	9.9	11.7	8.5	10.8
24 hours.....	17.0	16.9	13.0	11.6	12.7	12.5	12.8	12.8

as iron and aluminum phosphate is highly correlated with fixation. That the mechanism of fixation is precisely the same under purely laboratory conditions when mixing was done by mechanical shaking, also is evident from table 11. If, instead of treating the soils individually, the correlation is considered as a group and the fixation values of the soils are plotted against the recovery values, it will be seen that the phosphorus recovered as iron and aluminum phosphate is roughly equal to the phosphorus fixed.

### SUMMARY AND CONCLUSIONS

With a view to obtaining definite information regarding the nature of phosphates formed in soils through fixation of applied soluble phosphates, mixtures of soil and phosphatic fertilizers were incubated at the optimum moisture content and the samples fractionated at periodic intervals. The effect of kind and amount of fertilizer, length of contact period, and nature of the soil on the amount, rate, and nature of phosphatic fixation has been studied in detail. The fertilizers were bonemeal, Nicifos 2/1, and Nicifos 4, each used at two concen-

trations on two unmanured soils from Dacca and Berhampur. Time of contact varied from immediate to 6 weeks. The following are the findings in brief:

As the amount of phosphorus added increased the available phosphorus also increased but not in the same proportion. As the time of contact between the phosphorus compound and the soil increased, the available phosphorus tended to decrease for 2 weeks then remained nearly constant.

As the amount of phosphorus added increased, the amount of phosphorus fixed also increased, though not in the same proportions.

Forty-five to eighty-five per cent of the added phosphorus was fixed at the start of the incubation. As the contact period increased to 6 weeks, these figures rose to 70 and 95 per cent respectively.

The percentage of  $P_2O_5$  fixed at any time was lower at a higher rate of application.

For similar rates of application, phosphorus of bonemeal was less readily fixed than that of Nicofos.

The amount of phosphorus fixed in the different treatments was highly correlated with the phosphorus recovered as iron and aluminum phosphates. More than 90 per cent of the fixed phosphorus was recovered as iron and aluminum phosphates, showing that chemical precipitation of soluble phosphorus into phosphates of iron and aluminum accounts very largely for the fixation that takes place in acid soils.

No phosphorus reverted to apatite form.

The nature and mechanism of fixation in the Dacca and Berhampur soils are precisely the same, though in the former soil fixation took place to a much less degree. In the Dacca soil, bonemeal appeared to be much superior to Nicifos so far as fixation was concerned.

In order to obtain a fuller understanding of the effect of time on the rate of fixation, experiments were carried out at short-time intervals by shaking the soil with solutions of known  $P_2O_5$  content for periods ranging from a few minutes to 24 hours. Following are the results:

Fixation took place immediately on mixing the soil with the fertilizer solution. It regularly increased with time for 24 hours, though for a period of 6 hours the rate of fixation was very rapid.

That the mechanism of fixation was the same under these conditions and in the incubation experiments is evident from the fact that here, as well, the amounts of phosphorus fixed by different soils were almost fully recovered in the corresponding fractions of iron and aluminum phosphate.

#### REFERENCES

- (1) BURD, J. S., AND MURPHY, H. F. 1939 The use of chemical data in the prognosis of phosphate deficiency in soils. *Hilgardia* 12: 323-340.
- (2) CHANDLER, W. V. 1941 Phosphorus adsorption by five Alabama soils as influenced by reaction, late saturation and free sesquioxides. *Jour. Amer. Soc. Agron.* 33: 1-12.
- (3) DEAN, L. A. 1938 An attempted fractionation of the soil phosphorus. *Jour. Agr. Sci.* 28: 234-244.
- (4) FORD, M. C. 1933 The nature of phosphate fixation in soils. *Jour. Amer. Soc. Agron.* 25: 134-144.
- (5) GHANI, M. O. 1943 Fractionation of soil phosphorus: 1. Method of extraction. *Indian Jour. Agr. Sci.* 13: 29-45.
- (6) GHANI, M. O., AND ALEEM, S. A. 1943 Studies on the distribution of different forms of phosphorus in some Indian soils: I. Surface distribution. *Indian Jour. Agr. Sci.* 13: 283-288.

- (7) GHANI, M. O. 1943 The use of 8-hydroxy quinoline as a means of blocking active iron and aluminum in the determination of available phosphoric acid of soils by dilute acid extraction. *Indian Jour. Agr. Sci.* 13: 562-565.
- (8) GILE, P. L. 1933 The effect of different colloidal soil materials on the efficiency of superphosphate. U. S. Dept. Agr. Tech. Bul. 371.
- (9) HECK, A. P. 1934 Phosphate fixation and penetration in soils. *Soil Sci.* 37: 343-355.
- (10) HIBBARD, P. L. 1935 Factors influencing phosphate fixation in soils. *Soil Sci.* 39: 337-358.
- (11) MATTSON, S. (1931) The laws of soil colloidal behavior: VI. Amphoteric behavior. *Soil Sci.* 22: 343-365.
- (12) METZGER, W. H. 1941 Phosphorus fixation in relation to iron and aluminum of the soil. *Jour. Amer. Soc. Agron.* 33: 1093-1099.
- (13) PUGH, A. J., AND DU TOIT, M. S. 1936 The composition and ionic exchange of ferric silicates and phosphates. *Soil Sci.* 47: 417-431.
- (14) RAVIKOVITCH, S. 1934 Anion exchange: I. Adsorption of the phosphoric acid ions by soils. *Soil Sci.* 38: 219-239.
- (15) ROSEMAN, C. A. 1927 Retention of phosphorus by soil colloids. *Soil Sci.* 24: 465-474.
- (16) ROYCHOWDHURY, S. P., AND MUKHERJI, M. K. 1941 Studies on Indian red soils: II. Fixation of phosphates. *Indian Jour. Agr. Sci.* 11: 205-219.
- (17) SCARSETH, G. D., AND TIDMORE, J. W. 1934 The fixation of phosphates by clay soils. *Jour. Amer. Soc. Agron.* 26: 152-162.
- (18) SCARSETH, G. D. 1935 The mechanism of phosphate retention by natural aluminosilicate colloids. *Jour. Amer. Soc. Agron.* 27: 596-616.

# OCCURRENCE, NATURE, AND LAND-USE SIGNIFICANCE OF "SILTPAN" SUBSOILS IN WEST VIRGINIA<sup>1</sup>

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The term "hardpan" or "siltpan" is commonly used in this region to describe certain lower subsoil horizons showing gray, brown, and yellow mottling. These horizons occur in soil profiles on level areas or on gentle upland or terrace slopes where there is no evidence of a persistent high water table. The term "hardpan" is based on the fact that the typical horizon is rather harsh and dense, is difficult to penetrate with an auger in dry weather, and is resistant to picking or breaking when removed in lump form or when studied in place. In all cases, a lump of the material will soften quickly in water to form a nonplastic or only slightly plastic mass. There is no evidence of protrusion of this layer in road cuts or excavations as described for certain hardpan horizons (8, 9, 15). The physical hardness and resistance may easily be overlooked when the soil profile is thoroughly wet.

## DISTRIBUTION OF SILTPAN SOILS

Siltpan subsoils occur throughout most of West Virginia over a considerable range of temperature, rainfall, and elevation. Well-developed pans occur in the Ohio River basin on old alluvial terraces at elevations of less than 700 feet where the mean annual temperature is 56 to 58°F. and the annual rainfall about 40 inches. At another extreme, siltpans are found at elevations of about 2,500 feet, where the mean annual temperature is 49 to 50° and the annual rainfall 48 to 52 inches. This condition prevails on ridgetops throughout the higher Appalachian Plateau region and in the higher limestone valleys of the southeastern ridge and valley portion of the state (Greenbrier Valley, for example). Siltpans also occur in the eastern valleys at elevations as low as 500 feet, mean annual temperatures of 52 to 55°, and annual rainfall as low as 30 inches. The fact that this type of subsoil is recognized and mapped under such varying climatic conditions suggests that it is a horizon of considerable significance throughout this entire region.

## PARENT MATERIALS OF SILTPAN SOILS

As indicated by Jenny (6), it is often impossible to be sure that the material underlying a particular soil profile actually represents the parent material from

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which the soil profile has developed. Even so, there usually seems to be a fair basis for assumptions as to the probable nature of the soil material at zero time, as parent material is defined by Jenny (6). Such assumptions, in the cases of hardpan or siltpan soils, invariably indicate that the parent material was relatively low in clay. Either silt or sand (especially fine sand) might have been predominant, but apparently never clay. Silty shales or fine-grained sandstones on the upland and stratified silt and sand in terrace deposits seem to be major sources of siltpan horizons. Clays are often interbedded with the sands or silts on the terraces. Light-weight siltstone material which presumably was rich in free carbonates is evidently the parent material in some cases in the limestone valley areas.

#### SOME PHYSICAL PROPERTIES OF PANLIKE SUBSOILS

The color of panlike horizons is invariably rather light. Field descriptions usually indicate yellow as the predominant color, with some gray and brown mottling. Although there is considerable range in the color shades and in the degree of mottling, the general color pattern is relatively consistent. Brown is seldom predominant and it may be lacking.

Air-dry colors in the laboratory may be matched fairly satisfactorily against color standards (12). Light yellowish brown, pale yellow, weak yellow, yellowish white, light gray, and light brown include all colors that have so far been observed in pan horizons (table 1).

Siltiness is commonly the first impression of the texture as observed in the field. Close examination, however, and thorough working of the material reveal a range in texture from fine sandy loam to silty clay, as judged by field men. Stickiness or plasticity is never more than moderate. Laboratory measurements thus far show silt contents ranging from 22 to 42 per cent, and clay from 14 to 40 per cent (table 1).

Examination of panlike horizons in place or in large lumps reveals either a grossly massive structure or distinct lamination, usually from  $\frac{1}{8}$  to  $\frac{1}{2}$  inch in thickness (figs. 1 and 2). In either case, many encrustations or vesicular openings are evident, usually with slightly darker interior than is common in the ground mass. The laminated type of structure is most common with material that shows some stickiness when wet.

Easy slaking and dispersion characterize panlike subsoils (figs. 3 and 4). If air-dry lumps are dropped into water, virtually all of the material will quickly pass a 0.5-mm screen. Gentle elutriation of seven typical samples varying in clay content showed between 26 and 57 per cent of the samples in the 1.0 to 0.1 mm. size range when no dispersing agent was used. The 57 per cent was obtained with a sample having a high clay content, and with this sample only 2 per cent remained in the 1.0 to 0.1 mm. size range after standard dispersion with sodium oxalate and mechanical stirring (11). Oxalate without stirring gave almost as complete dispersion, and stirring<sup>a</sup> without a dispersing agent gave simi-

<sup>a</sup> Stirring for 10 minutes in a drink mixer.

TABLE 1  
*Some physical characteristics of several subsoils, including siltpan horizons*

SAMPLE NUMBER	DEPTH	NATURE OF HORIZON	SOIL	MOISTURE EQUIVALENT	CLAY*	SILT*	DOMINANT COLOR†
				per cent			
1a	inches	Upper subsoil Laminated siltpan Parent siltstone	Pickaway silt loam	18.9	35.0	per cent	Light yellowish brown
	8-18			26.5	40.0		
	18-41			16.2	13.7		
2a	8-22	Upper subsoil	Pickaway silt loam	18.6	16.0	per cent	Light yellowish brown
	22-39	Laminated siltpan		18.5	21.5		
	39+	Parent siltstone		16.6	15.6		
3a	15-21	Upper subsoil	Monongahela loam	15.8	14.0	per cent	Light brown to light yellowish brown
	25-30	Massive loamy pan		16.0	14.0		
	35-40	Massive loamy pan		12.0	8.6		
4a	20-25	Upper subsoil	Cookport	22.1	16.0	per cent	Light yellowish brown
	28-32	Massive loamy pan		22.0	30.4		
	35-40	Fine weathered sandstone		27.3	27.1		
5a	12-18	Upper subsoil	Monongahela fine sandy loam	18.1	v.low	per cent	Light gray
	24-36	Massive sandy pan		12.6			
	40	Sand + gravel					
6a	10-18	Upper subsoil	Monongahela silt loam	14.8	low	per cent	Light yellowish brown
	18-30	Massive sandy loam pan		19.2			
	30-40	Clay		30	v.high		
7a	12-23	Upper subsoil	Silty terrace, prob. wind-laid over water-laid	20.6	per cent	per cent	Pale yellow to weak yellow
	23-28	Massive siltpan		19.6			
	28-46	Sandy clay		22.5			
8a	10-18	Upper subsoil	Shallow Cookport	25.9	per cent	per cent	Light yellowish brown
	22-28	Shaly siltpan		24.9			
	32-36	Weathered parent shale					
9a	16-22	Upper subsoil	Shallow Cookport	25.3	per cent	per cent	Light yellowish brown
	26-32	Shaly siltpan		24.7			
	36+	Weathered parent shale					

\* By the standard pipette method (11).

† Air-dry color by color standards (12).

lar results, indicating weak aggregate stability. With increasing sandiness and decreasing clay the difference between dispersed and nondispersed treatments

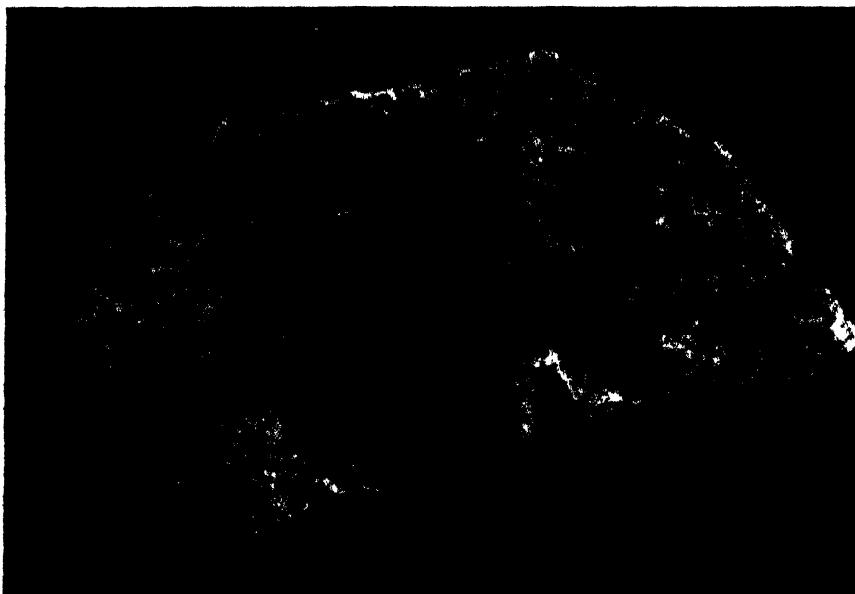


FIG. 1. A NATURAL LUMP OF A TYPICAL, GROSS-MASSIVE SILTPAN SUBSOIL  
Note large encased pores or vesicular openings (slightly enlarged)



FIG. 2. A NATURAL LUMP OF A TYPICAL LAMINATED SILTPAN SUBSOIL  
Note horizontal cleavage lines and encased pores

decreased. The extreme of sandiness showed 45 per cent between 1.0 and 0.1 mm. with no dispersion except gentle elutriation, and 42 per cent in the same size range after thorough dispersion. This indicates only 3 per cent of aggrega-

tion into sand-sized units compared to the 55 per cent obtained when the clay content was high. Evidently clay is the main aggregating agent, but all aggregates are small and weakly held. The impression of siltiness often gained in field observations can either be due to a high content of silt and fine sand textural units or to clay-formed aggregates in that size range. (See table 2 for actual contents of dispersed clay and silt.)

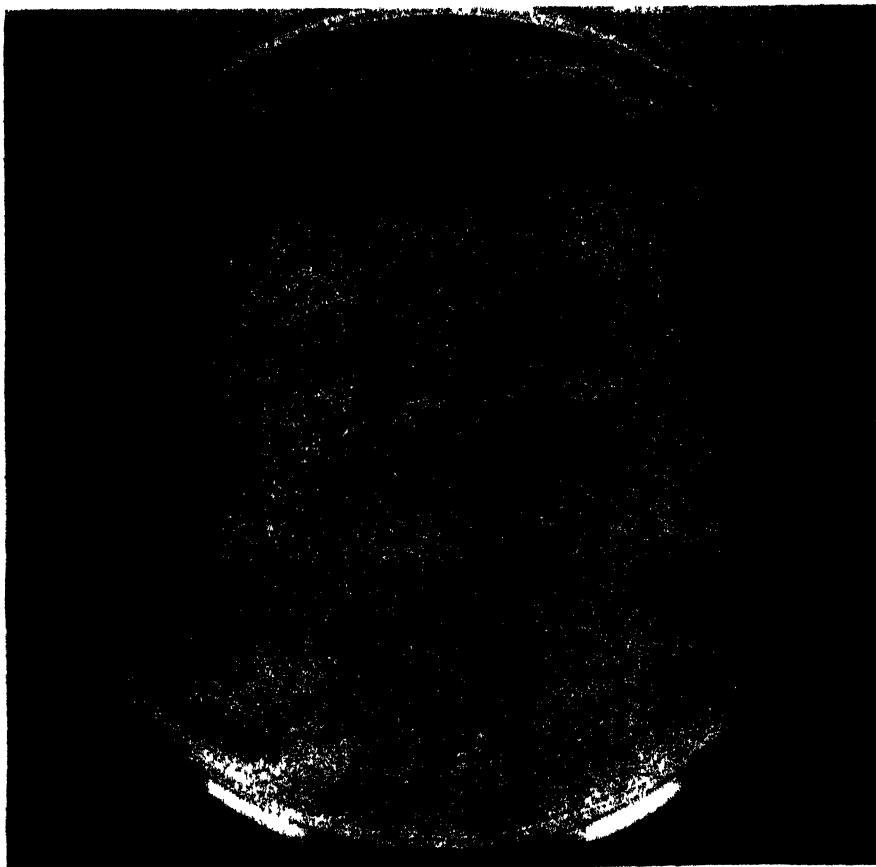


FIG. 3. A STRONGLY DEVELOPED SILTPAN SLAKED IN WATER AND ALLOWED TO DRY IN A UNIFORM LAYER

Note that the structure is essentially that of closely packed silt or fine sand particles.  
Compare with figure 4

Another feature of panlike horizons is their apparent tendency to hold only relatively small quantities of water. Several factors are probably responsible for this. The volume weight is invariably high (1.55 to 1.72 for varied samples determined). Thus the total pore space is relatively low (approximately 35 to 42 per cent). In addition, the density and lack of coarse openings for water cause relatively slow rewetting following thorough drying. Then there is also probably considerable trapping of air in the pore size range represented (13),

and therefore this horizon seldom reaches full saturation. After laboratory wetting and percolation measurements, panlike samples usually contain less than 30 per cent moisture by volume, and sometimes as little as 20 per cent. Even laboratory evacuation and wetting seldom bring the moisture contents to within 5 per cent of the calculated values for pore space, apparently indicating some completely nonfunctional porosity.

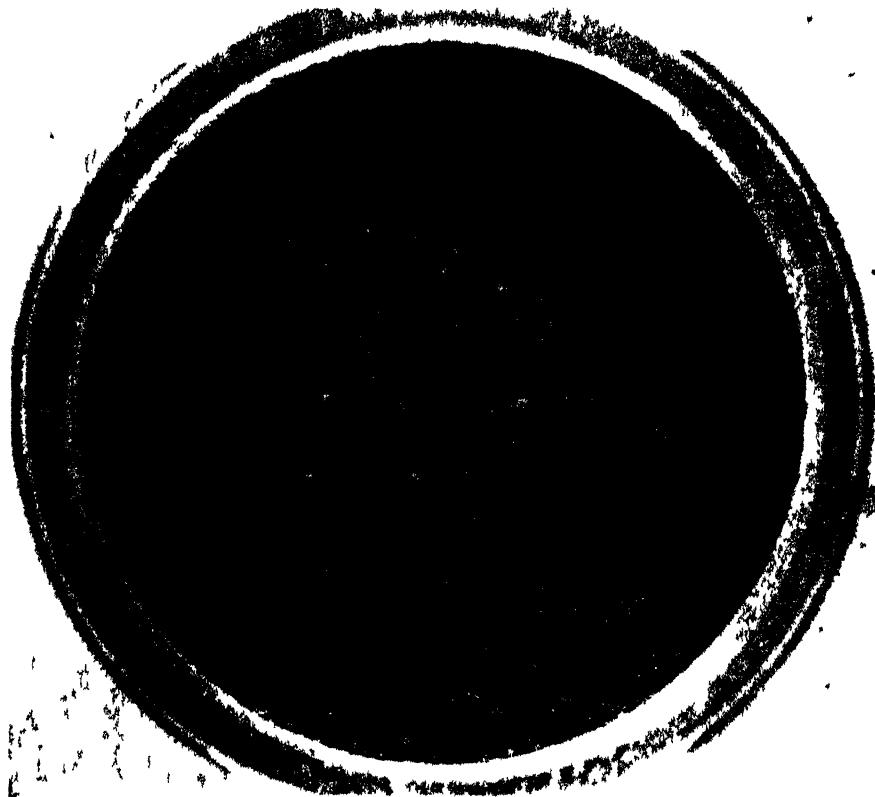


FIG. 4 A SILTY SUBSOIL WITH NORMAL NUTLIKE STRUCTURE SLAKED IN WATER AND ALLOWED TO DRY IN A UNIFORM LAYER

Note that it shows considerably more aggregation and porosity than the siltpan subsoil of figure 3

Moisture equivalents (table 1) are all rather low but vary considerably with texture. The lack of high values reflects the relatively light textures of all pan materials.

#### SOME CHEMICAL PROPERTIES OF PANLIKE HORIZONS

Table 2 shows that the various subsoil panlike horizons differ considerably in simple acid-base properties, but seem to lie within reasonably well-defined limits. The pH values are commonly between 4.6 and 5.0, with measured extremes of 5.1 and 4.3. A number of other pH values determined but not listed

have all fallen within these extreme limits. Exchangeable bases are consistently rather low. Exchange capacity is variable but never high. The percentage

TABLE 2  
*Some chemical characteristics of several subsoils, including siltpan horizons*

SAMPLE NUMBER	pH	TOTAL BASES BY KAPPEN METHOD (7) m.e./100 gm.	TOTAL EXCHANGEABLE BASES* m.e./100 gm.	EXCHANGE CAPACITY* m.e./100 gm.	BASE SATURATION* per cent	EXCHANGEABLE POTASSIUM† m.e./100 gm.	POTASSIUM SATURATION per cent	ORGANIC MATTER‡ per cent
1a	5.25	6.8						0.75
b	5.1	4.2	2.2	11.1	19.8	.20	1.8	0.25
c	5.0	2.0	0.8	3.7	21.6	.06	1.6	0.45
2a	5.3	2.9	2.6	7.0	37.1	.20	2.8	0.40
b	5.1	3.2	2.4	9.7	26.8	.19	2.0	0.18
c	5.1	3.1	0.8	4.3	19.0	.10	2.3	0.11
3a	4.85	3.8	2.2	5.4	40.7	.12	2.2	0.12
b	4.7	3.7	2.0	6.7	29.8	.13	2.0	0.10
c	4.8	5.2	3.3	8.1	40.7	.18	2.2	0.13
4a	4.6	6.8	2.2	9.2	23.9	.28	3.0	0.36
b	4.6	7.8	2.9	10.0	29.0	.24	2.4	0.31
c	4.6	9.2						0.26
5a	4.5	4.4				.06		0.22
b	4.3	1.5				.14		0.13
c	4.3							
6a	5.0	5.5	3.1	4.9	63.3	.12	2.5	0.31
b	4.9	6.9	3.2	6.5	49.2	.14	2.2	0.29
c	4.8	15.3	9.9	20.1	49.2	.57	2.8	
7a	5.6	7.9	7.0	9.5	73.6	.32	3.3	0.31
b	5.1	6.0	2.7	9.2	29.3	.18	2.0	0.26
c	5.3	8.7	7.7	9.5	81.1	.27	2.8	0.20
8a	5.1	7.3	4.2	11.3	37.2			0.55
b	5.0	6.1	4.0	11.1	36.0	.24	2.2	0.38
c	5.0	6.0	3.4	9.8	34.7	.24	2.3	
9a		6.0				.28		0.40
b	4.9	6.5				.34		0.20
c		6.5						0.20

\* Determined by a slight modification of the neutral acetate method (1).

† Determined by method of Brown *et al.* (2).

‡ Determined by the modified Walkley-Black method (3).

base-saturation varies from about 20 to 40. Sample 6 has a higher value (49 per cent), which is probably associated with the fact that the pan layer is here underlain by a heavy clay with rather high base content. One of the most con-

sistent properties measured is the percentage saturation with potassium. This is close to 2.0 per cent in the samples determined and, like most of the other properties, is more consistent for the several pan layers than it is within each soil profile (table 2).

Organic matter values are all low, as would be expected at the depths represented. The overlying horizons are somewhat higher in all cases, and there is little difference between the pans and lower layers.

#### DISCUSSION

Under West Virginia conditions it is clear that the so-called "hardpan" or "siltpan" horizons are not such strongly cemented layers as described elsewhere (8, 9, 10, 15). They apparently correspond with the "Y" horizon of Bushnel (4). The firmness and harshness, evident when the material is dry, is primarily the result of the high density of packing and the complete lack of any effective aggregation. The gross structure is either massive or laminated. Coarse, visible pores are represented by encased channels or vesicular openings which are discontinuous and probably contribute little to the effective porosity. When laminations occur, the material is essentially massive within each platelike block. Distinct laminations are usually spaced at from  $\frac{1}{8}$ - to  $\frac{1}{2}$ -inch intervals, and the plates thus defined are firm and harsh like the completely massive pan.

Siltpan horizons may be slow to wet, but they are never impervious to water. Laboratory percolation rates are slow, but vary with the texture and are not extreme, as in massive clays. The range of percolation rates for natural lump samples is between 0.1 and 0.01 inch per hour by the methods used.<sup>4</sup> Those for massive clays are usually from 0.01 to 0.001 inch per hour.

The siltpan subsoil is always a zone of strong leaching. This is indicated by pH values as well as the low base and base-saturation levels. In fact, it is believed that iron, too, is being or has been removed. The coloration of the pan horizon and of the soil profile above is invariably weak, and concretions or agglomerations of iron are rare.<sup>5</sup> Discolorations or mottling within the pan appears to be due primarily to the differential removal of iron oxides and other coloring agents rather than to iron reduction by lack of aeration. The light-colored portions can often be identified under a microscope as clean quartz sand or silt.

Total and exchangeable bases, and degree of base and of potash saturation are often lower than in the horizons above or below. This seems to indicate that the pan layer is likely to be more strongly leached of certain elements than any other portion of the soil profile. In the case of the layer below, its greater depth and closer relation with the parent rock would account for a different or a lesser degree of leaching. For layers above, the factors of enrichment by surface litter and of soil structure are probably involved. Enrichment by the litter is apparently confined primarily to the soil profile above the pan layer. This is true also of the major aggregating factors—organic matter, roots, freezing, and drying.

<sup>4</sup> Percolation rate with a hydraulic head equal to the sample length (14).

<sup>5</sup> A few occur in sample 6, where bases are rather high.

In the upper zone where these factors are effective, the leaching waters probably move downward primarily through channels or along structural planes. Below this zone, where the pan occurs, the water moves slowly through the massive material. Thus, both the factor of time and of more complete contact might account for stronger leaching of the panlike horizon than of upper layers in the same soil profile. A more continuous and more complete hydration, and possibly greater reducing action, might also contribute to the strong leaching of this lower layer.



FIG. 5 MONONGAHELA SILTPAN SUBSOIL (LEFT) SUPPORTS ABOUT THE SAME GROWTH OF SUDAN GRASS AS UPSHUR CLAY SUBSOIL (RIGHT) WHEN BOTH ARE LIMED TO pH 6.5 AND LIBERALLY FERTILIZED (PKN) IN THE GREENHOUSE  
The growth shown was almost as good as that on any of three other subsoils

Recognizable siltpans are a good index of a particular soil condition but are not necessarily a seriously detrimental feature in themselves. Even if the pan layer could be completely removed, the remaining soil would have rather limited capabilities. The condition represented is evidently very widespread in the region, but varies greatly in degree. A trace of the pan layer can be found in most silty or fine sandy soils of sufficient depth. Strong and well-defined siltpans occur primarily on slopes of 5 per cent or less, where they indicate a very strongly leached or "old" silty or fine sandy soil profile. Bases and organic matter are always low in the surface soil as well as the subsoil, and the surface soil structure approaches a single-grained condition. As a result, surface crusting occurs on cultivated land, and severe erosion is the rule even on relatively gentle slopes unless the soil surface is continually protected by close-growing crops or organic mulches.

Such practices as pan breaking are futile on such soils because the panlike horizon is only a symptom of the soil profile condition and is not the primary limitation. Some benefit might be derived from deep placement of lime and fertilizer along with pan breaking, but without adequate fertility, the physical disruption would not seem worthwhile. In greenhouse trials as well as in the field, siltpan soils give good response to lime and phosphorus and to potash. No evidence of other extraordinary deficiencies have been noted (fig. 5).

A further special importance of the siltpan horizon has been indicated by Galpin (5) in the case of soil slips in colluvial materials. His studies indicate an unstable horizon, high in effective silt particles, which is the main source of weakness in certain soil slips. The present studies seem to indicate that this horizon corresponds with the well-known siltpan horizon occurring on gentle slopes. Hillside slipping and creep tend to prevent some of the firmness that leads to the recognition of a true pan layer, but the colorations, physical instability in water, degree of leaching, and depth within the soil profile are markedly similar.

#### SUMMARY AND CONCLUSIONS

Lower subsoil horizons known as "siltfans" are widely recognized throughout West Virginia on silty or fine sandy parent materials at elevations varying from about 500 feet to at least 2,500 feet. Pans are light colored, and mottled with gray, brown, and yellow. The gross structure is massive or laminated, and the grains are densely packed to give high volume weights, with all coarse pores disconnected and usually encased with brown coatings. All pans slake readily in water and have low plasticity. Textures range from fine sand to silty clay. Water permeability is low but variable with the texture and always appreciable. Total porosity and water capacities are low, and dry pans tend to re-wet slowly. The pH values are usually between 4.6 and 5.1; base saturation 20 to 40 per cent; total bases, exchange capacity, and moisture equivalents medium to low. Potash saturation was close to 2 per cent in all of seven determinations made. Siltfans in West Virginia are apparently never strongly cemented layers, are always strongly leached, and occupy a position between the upper horizons, where definite soil structural features have developed, and the lower horizons, where parent material characteristics predominate. The degree of differentiation of this horizon is a good index of soil leaching and of the physical and chemical limitations of the soil profile. Adequate liming and fertilizing of pan horizons permits satisfactory growth in the greenhouse, but in the field poor structure, low water reserves, low organic matter, and extreme erodibility of the upper profile combine with low mineral fertility to restrict the use of soils with strong siltpan development. Pan breaking cannot be effective because this would not remove the limitations mentioned. On colluvial slopes the siltpan horizon seems to represent the zone of weakness in many soil slips.

#### REFERENCES

- (1) BRAY, R. H., AND WILHITE, F. M. 1929 Determination of the total replaceable bases in soils. *Indus. and Engin. Chem., Analyt. Ed.* 1: 144.

- (2) BROWN, D. S., ROBINSON, R. R., AND BROWNING, G. M. 1938 Determination of small amounts of potassium. *Indus. and Engin. Chem., Analyt. Ed.* 10: 652-654.
- (3) BROWNING, G. M. 1938 A comparison of the dry combustion and the rapid titration method for determining organic matter in soil. *Soil Sci. Soc. Amer. Proc.* 3: 158-161.
- (4) BUSHNELL, T. M. 1940 An outline of the classification of Indiana soils. *Proc. Ind. Acad. Sci.* 49: 151-158.
- (5) GALPIN, S. L. 1940 "Blister" slips in West Virginia. *Soil Sci. Soc. Amer. Proc.* 5: 418-420.
- (6) JENNY, H. 1941 Factors of Soil Formation. McGraw-Hill Book Co., New York.
- (7) KAPPEN, H. 1929 Die Bodenazidität. Julius Springer, Berlin.
- (8) KRUSEKOPF, H. H. 1942 The hardpan soils of the Ozark region. *Soil Sci. Soc. Amer. Proc.* 7: 434-436.
- (9) MARBUT, C. F. 1935 Soils of the United States. Atlas of American Agriculture, Part III. U. S. Dept. Agr., Washington, D. C.
- (10) NIKIFOROFF, C. C. 1941 Hardpan and microrelief in certain soil complexes of California. U. S. Dept. Agr. Tech. Bul. 745.
- (11) OLMISTEAD, L. B., ALEXANDER, L. T., AND MIDDLETON, H. E. 1930 A pipette method of mechanical analysis of soils, based on improved dispersion procedure. U. S. Dept. Agr. Tech. Bul. 170.
- (12) RICE, T. D., et al. 1941 Preliminary color standards and color names for soils. U. S. Dept. Agr. Misc. Pub. 425.
- (13) SMITH, R. M., AND BROWNING, D. R. 1942 Persistent water unsaturation of natural soil in relation to various soil and plant factors. *Soil Sci. Soc. Amer. Proc.* 7: 114-119.
- (14) SMITH, R. M., BROWNING, D. R., AND POHLMAN, G. G. 1944 Laboratory percolation through undisturbed soil samples in relation to pore size distribution. *Soil Sci.* 57: 197-213.
- (15) WINTERS, E. 1942 Silica hardpan development in the red and yellow podzolic soil region. *Soil Sci. Soc. Amer. Proc.* 7: 437-440.



# CHANGES PRODUCED IN KAOLINITE BY DRY GRINDING

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Many investigators (4, 5, 6, 8, 9, 10, 13, 14) have made studies with ground clays or ground soil colloids. With few exceptions, all have assumed that dry grinding does not produce a chemical change in the ground material; that is, when kaolinite is ground, the fine material is still kaolinite. Nearly all investigators have found that grinding a clay mineral increases its base-exchange capacity, but there is no general agreement as to the correct explanation for this increase. Kelley and co-workers (8, 9) are of the opinion that the increase in exchange capacity is due to fracture of the crystals, which produces more edges, and that the broken bonds on these edges hold easily replaceable hydrogen ions. Shaw (13) has shown that wet grinding of kaolinite causes shearing along the cleavage planes, whereas dry grinding fractures the crystals. He reported that the dry-ground material exhibited a higher exchange capacity than the wet-ground material. He interpreted this to mean that the exchange positions are on the crystal edges and that the increase in exchange capacity was due to an increase in the number of edges, a view which is in agreement with that of Kelley and co-workers. Stout (14) attributed the increase in exchange capacity to shearing along the cleavage planes, which would expose more "surfaces" with OH groups. None of these investigators suggested the formation of new compounds as a result of dry grinding, but all assumed that they were working with smaller particles of the original material. Marshall (10) questioned the validity of such an assumption. He suggested that fine grinding alters the composition of the original material and that there may be present in the final product one or more new mineralogical entities. He concluded that fine grinding not only cleaves the crystals in their planes of weakness, but also distorts every part of the lattice; that the exchange capacity of the ground material will depend upon the crystal structure of the new material and need not bear any simple relationship to the original. Jackson and Truog (4) would seem to agree with Marshall.

Naturally, the validity of various interpretations regarding ground clays hinges on a knowledge of what happens during the grinding process. There is insufficient evidence at hand to decide the issue. The problem is of more than academic interest, since some of our theories of the mechanism of the base-exchange reaction are based upon studies made with ground material. It is also quite probable that many clays closely resemble artificially ground mineral specimens. This investigation was conducted to throw additional light upon this controversial subject.

## EXPERIMENTAL PROCEDURE

The kaolinite used throughout this investigation was packed by Merck and Company and sold as "Merck's Colloidal Kaolin" (original source unknown).

TABLE 1  
*Mechanical analysis of original kaolinite sample studied*

PARTICLE SIZE	PERCENTAGE OF SAMPLE
$\mu$	
>5.0	1.3
5.0 -1.0	31.7
1.0 -0.5	25.5
0.5 -0.25	32.1
0.25-0.125	4.5
<0.125	4.9

TABLE 2  
*X-ray data for original kaolinite sample studied*

I*	d	II†	d	III‡
Intensity§	d	Intensity	d	d
	A.		A.	A.
m	7.90			
s	7.17	s	7.20	7.14
m	4.61	w	4.45	4.45
m	4.41	vs	4.33	4.397
w	4.20			4.248
s	3.86	s	3.51	3.776
m	2.82			3.510
w	2.786			2.784
w	2.778			
vw	2.500	w	2.49	2.480
vw	2.374			2.380
vw	2.333	s	2.300	2.343
w	2.240			2.242
vw	2.220	s	2.220	2.225
vw	2.205			2.200
m	2.100	vw	1.970	2.098
		vw	1.860	1.969
		m	1.650	1.854
		m	1.470	1.483

\* Powder diagram made by M. L. Jackson, University of Wisconsin, who reported: "Pattern XRD1209 of unground kaolinite is typical of very pure kaolinite samples; in fact, it is as good a pattern as we have ever obtained from kaolinite" (personal communication).

† A 1 per cent suspension was allowed to dry down at room temperature. A small 1 cm. sq. "cake" of the settled kaolinite was x-rayed with the usual technique for producing Laue' patterns. This work was done in the laboratory of the mineralogy department of Ohio State University.

‡ The theoretical lines given by Gruner (3).

§ m, moderate; s, strong; w, weak; vw, very weak. (All estimated visually.)

Although the sample is composed of extremely fine particles, as shown by the data in table 1, the sharp hexagonal crystals in figures 1A and 1B and the electron diffraction pattern (fig. 1D) indicate that the sample was not ground before packing (2, 12). The x-ray data are presented in table 2.

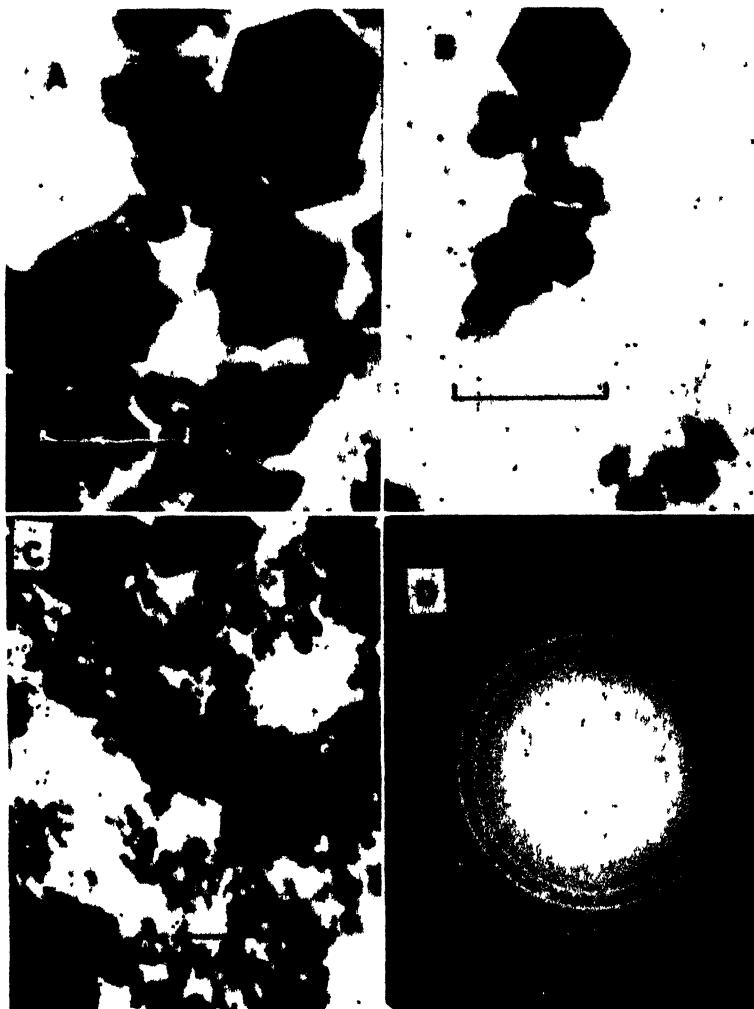


FIG. 1 A AND B, UNGROUND KAOLINITE; C, KAOLINITE GROUND DRY 96 HOURS; D, ELECTRON DIFFRACTION PATTERN OF UNGROUND SAMPLE

The line in A, B, and C represents 1  $\mu$

Individual 50-gm. samples were ground 24, 48, 72, 96, 144, and 192 hours in a porcelain ball mill with small stream-rounded granite pebbles serving as balls. Small specimens of the samples were studied with the electron microscope. The particle size distribution was determined by the modified pipette method. The exchange capacity was determined by leaching with normal neutral ammonium

acetate, and the adsorbed ammonia, by distillation with MgO. The exchanged bases were determined in the leachate, sodium as zinc uranyl acetate, calcium as the oxalate volumetrically with potassium permanganate.

## RESULTS

### *Exchangeable bases*

The exchange capacity of the original unground kaolinite sample was 4.5 m.e. per 100 gm. The ammonium acetate leachate from this unground sample contained 1.34 m.e. of sodium and 0.91 m.e. of calcium per 100 gm. of clay, thus indicating approximately 50 per cent base saturations. The exchange data for the ground samples are plotted in figure 2C. The data show that the sodium and calcium released from the clay by leaching with normal neutral ammonium acetate decreased with increased grinding time. This entirely unexpected result seemed to indicate that the sodium and calcium, which certainly must have been released as the mineral was broken, were recombined or fixed in a nonexchangeable form, presumably by other products of grinding.<sup>1</sup> Additional evidence in support of this explanation was obtained when a few of the untreated samples were electrodialyzed. For example, 5 hours in the Bradfield cell at 110 volts reduced the pH of the unground sample from 6.4 to 3.8 and that of the sample ground 192 hours from 5.9 to 5.6; that of the sample ground 96 hours showed no reduction. This sample had a pH of 5.9 throughout the dialysis.

As a further check on these results the exchange capacity was determined in several different ways. The results are presented in figure 2A. Curve 1 is ammonia removable by distillation. Curve 2 is for total calcium and represents the calcium released by electrodialysis plus the calcium recovered later when these same electrodialyzed samples were treated with 0.5 N NaOH and 0.1 N HCl as discussed below. Curve 3 shows calcium removable by electrodialysis from Ca-saturated clay (saturated by leaching after grinding). Curve 4 shows K titration of thoroughly electrodialyzed ground samples.

A comparison of curves 1 and 3 in figure 2A and the data in table 3 shows that when the samples were leached with calcium chloride and electrodialyzed, the calcium removed by electrodialysis was not equal to the exchange capacity determined by the ammonium acetate method. For the unground samples the results by the two methods were very nearly the same but for the ground samples, curves 1 and 3 (fig. 2A) become farther and farther apart with increased grinding time. *These results, it seemed, could best be explained by postulating that grinding the kaolinite destroyed its structure completely and that the resulting amorphous silica and alumina groups and free bases recombined into some materials, presumably similar to a permutite, which was relatively stable.* To test this assumption, the ground samples were treated with 0.5 N NaOH, and some additional samples were treated with 0.5 N NaOH followed by washing with 0.1 N HCl. This was done in an effort to dissolve any free silica or alumina that might have been released by grinding. The exchange capacity of these treated sam-

<sup>1</sup> The decrease in exchangeable bases seems to rule out the likelihood of contamination from the mill, since the quantities of bases were lower after grinding than before.

ples was then determined by ammonium acetate leaching. The results are presented in table 3.

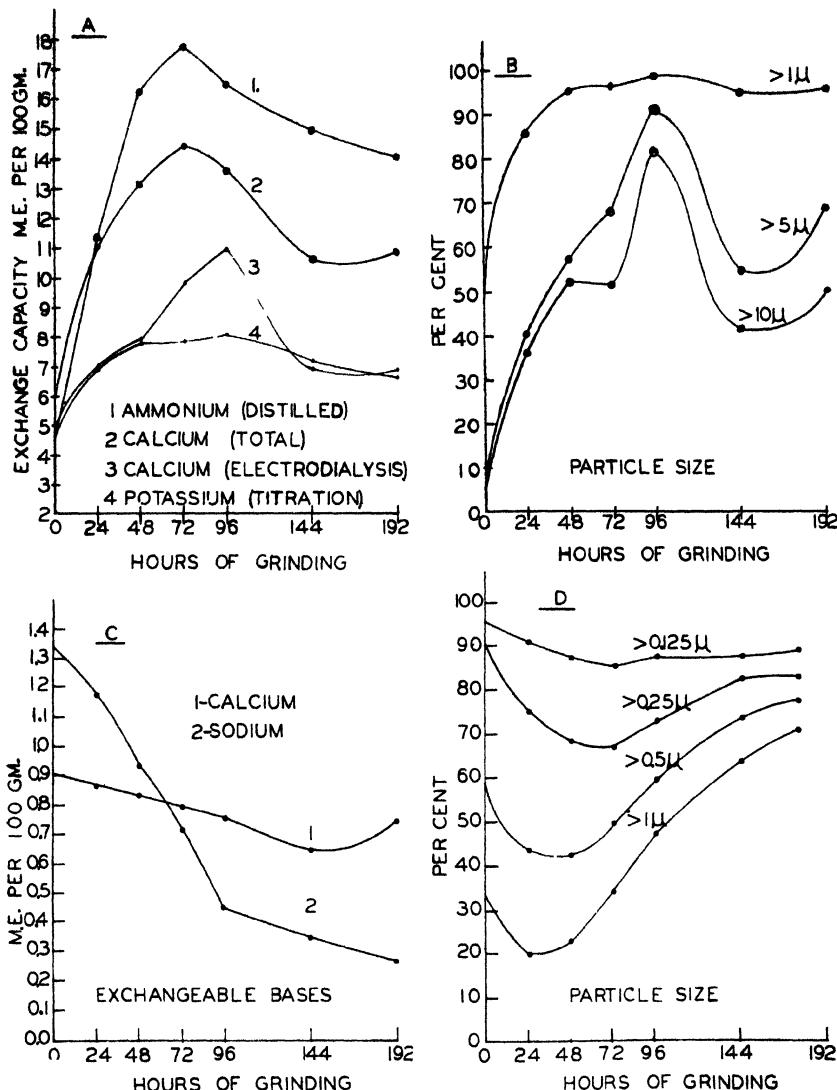


FIG. 2. EFFECT OF GRINDING KAOLIN UPON: A, EXCHANGE CAPACITY; B, PARTICLE SIZE; C, EXCHANGEABLE BASES. D, EFFECT OF 0.5 N NaOH ON THE PARTICLE SIZE OF GROUND KAOLIN

These data support the explanation and are interpreted as showing that with longer grinding more of the permutite-like material was present and thus more of the Ca was fixed. It will be noted that the exchange capacity increased up to 72 hours' grinding and then decreased. This might be expected if recombination does occur. This will be discussed more fully below. Column II, showing

exchange capacity after treatment with NaOH, supports the theory. It was expected that the NaOH treatment would serve to dissolve free silica released by grinding. Instead, it appears that the Na combined with the silica and alumina to form a permutite having the typical high exchange capacity. Upon treatment with weak acid (column III), this material was partly dissolved, resulting in a lowered exchange capacity.

As used in this discussion "permutite" means an apparently amorphous sodium alumina-silicate substance with a high base-exchange capacity, without reference to any definite silica-alumina ratio. The substance produced when ground kaolinite was treated with sodium hydroxide is designated a "permutite" because

TABLE 3

*Effect of grinding and various other treatments on the exchange capacity of kaolinite*

GRINDING TIME hrs	m.e. §	EXCHANGE CAPACITY		
		By ammonium acetate		
		I*	II†	III‡
0	4.88	4.5	5.2	3.24
24	7.01	11.3	10.3	3.31
48	7.83	16.2	12.6	5.79
72	9.82	17.7	44.1	7.53
96	10.95	16.5	125.4	11.27
144	6.87	15.0	180.6	45.08
192	6.91	14.1	219.2	30.45

\* Untreated samples.

† Treated with 0.5 N NaOH only.

‡ Treated with 0.5 N NaOH followed by washing with 0.1 N HCl.

§ m.e. = per 100 gm.

those properties studied were in close agreement with the properties of permutites as reported in the literature. The values obtained are in agreement with those reported for permutites (16, 17).

#### *Exchange capacity*

The data in table 3 and the curves in figure 2A show that the exchange capacity of the sample first increased and then decreased with increased grinding time. A hypothetical case may serve to explain this fact. Suppose it were possible to grind 100 gm. of kaolinite in a grinding apparatus so arranged that whenever the kaolinite crystals were changed to some material other than kaolinite, the new material would automatically be separated from the unchanged kaolinite. (Such changes might be produced by reduction to an extreme degree of fineness or distortion of the crystal lattice.) Then the total exchange capacity of the unchanged kaolinite in the apparatus would first increase as a result of increased number of broken edges, or increased "surfaces," but with longer periods of grinding it would decrease because there would no longer be 100 gm. of kaolinite

in the mill, since it would be removed as it was changed to some other material. The hypothetical curve 1, figure 5, represents this assumed effect.

On the other hand, the total exchange capacity of the material being formed, and automatically removed from the hypothetical apparatus, would increase with increased grinding time because more and more material would be formed. After long periods of grinding, the exchange capacity curve of this material would tend to level off because very little additional material would be formed with continued grinding since most of the kaolinite would have been used up. Hypothetical curve 2, figure 5, showed this effect.

Since it is not possible to use such a grinding apparatus, and both the unchanged kaolinite and the new material must remain mixed together during grinding, the exchange capacity of the mixture as found at any one time will obviously be the sum of the exchange capacities of the two materials present or will be represented by summation of curves 1 and 2. Curve 3, figure 5, is the summation of curves 1 and 2. The foregoing explanation is purely hypothetical; however, curve 3 compares very favorably with curve 4, which is the actual exchange capacity of the ground material as measured by the standard ammonium acetate method. The close agreement between these two curves supports the idea that the exchange capacity as determined in this experiment is for a mixture of materials and that grinding the kaolinite produced a permutite-like material.

The discrepancy in the exchange capacity as determined by electrodialysis of calcium-saturated clays and by titration of the electrodialyzed samples with potassium hydroxide (fig. 2A) is probably due to the fact that the former is for an end reaction, since Ca ions are removed from the solution, while the latter is for an equilibrium reaction, since none of the materials are removed from the solution. It might also be postulated that the difference is due to the different ionic sizes of calcium and potassium ions in relation to the adsorption forces and nature of the surface of the adsorbing material (11).

#### *Particle size*

The similarity in the shape of the curves in figures 2A and 2B also suggests some type of combination during grinding. The effective particle size and the exchange capacity increase together. This would not be expected if fracture of the crystals were all that took place during grinding, because, although fracture of the crystals might increase the exchange capacity because of increased number of broken edges, it would obviously decrease the particle size.

An explanation for this apparent contradiction is found again in the proposed formation of a new material which has been designated as a "permutite-like material" or a precursor of a permutite. With dry grinding the larger kaolinite crystals would be fractured across the crystal (13) and the finer ones would be reduced to such a degree of fineness that recombination would take place, producing a permutite-like material. Naturally this material accumulates on the flat surfaces of the plates, rather than the edges, thus adding to the thickness of the plates and causing it to settle with a larger size fraction. With continued

grinding the kaolinite crystals become completely coated with the products of grinding. The effect is the same as if a small disk or plate were rolled over and over in a paste until it became coated to resemble a sphere or ellipsoid. Naturally, further grinding after this state is reached fractures the crystal fragments which form the interior of the clumps. Thus the effective particle size

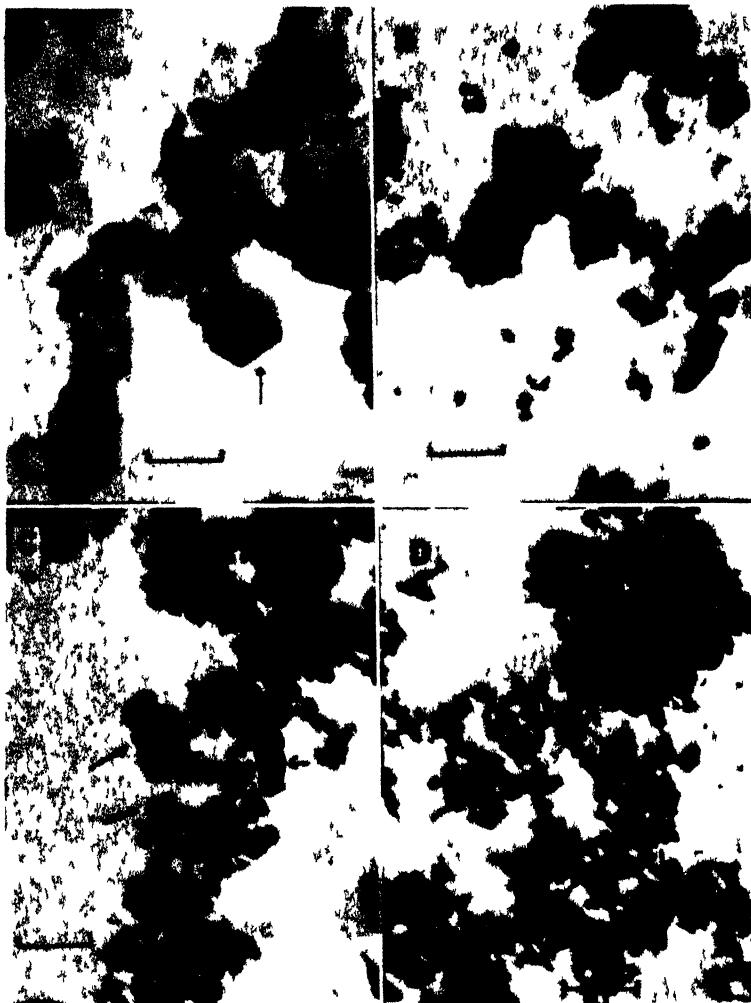


FIG 3 A, UNGROUND KAOLINITE, B, KAOLINITE GROUND 72 HOURS, C, KAOLINITE GROUND 96 HOURS, D, KAOLINITE GROUND 192 HOURS

Line represents 1  $\mu$

is decreased. Apparently, after 144 hours of grinding, the coated crystal fragments were reduced to such a size that continued grinding simply resulted in a "balling" together of the products of grinding and another increase in the effective particle size. The electron micrographs in figure 3 support this explanation. Figure 3A shows that many of the particles of unground kaolinite were thin

enough to have undergone electron penetration. In figure 3B the surface dimensions are of the same order of magnitude as that of the unground kaolinite but the particles are considerably thicker as shown by the fact that there is very little electron penetration. Figure 3C shows that there was no electron penetration of kaolinite ground for 96 hours, although it appears that many of the

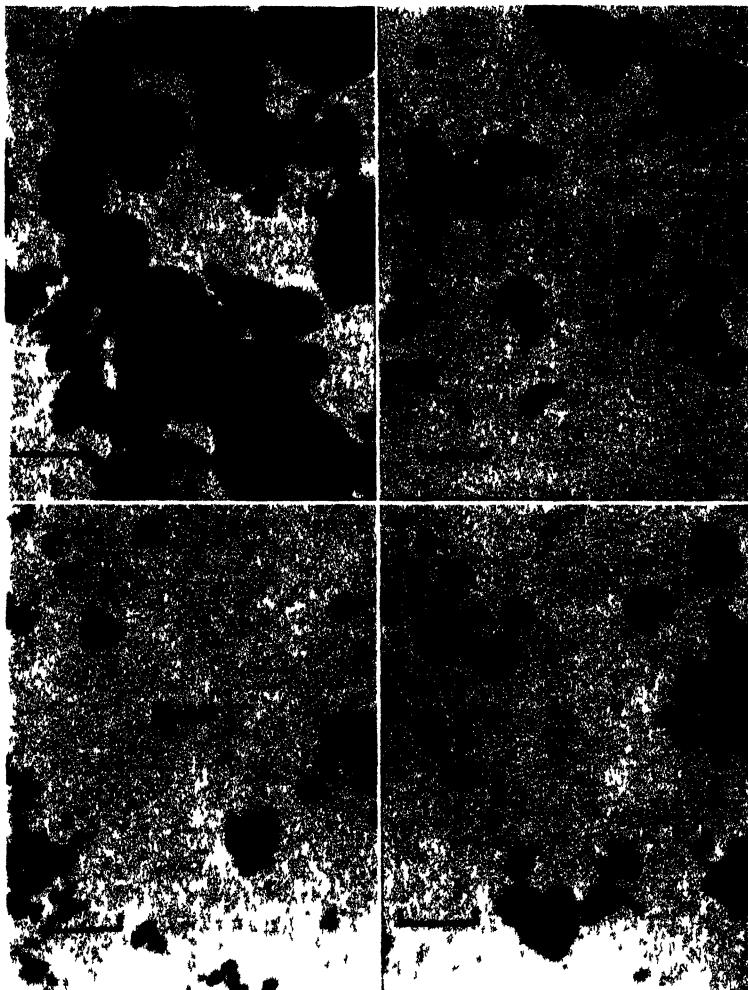


FIG. 4 KAOLINITE AFTER TREATMENT WITH 0.5 M NaOH A, UNGROUND; B, GROUND 72 HOURS, C, GROUND 96 HOURS, D, GROUND 192 HOURS  
Line represents 1  $\mu$

particles are crystal fragments completely surrounded by the products of grinding (arrows). In figure 3D there is little evidence of the presence of coated crystal fragments; even the large clump at the top is porous, as shown by the many small holes (white spots).

The curves in figure 2D, and figure 4 also support this explanation for the

effects of grinding upon the particle size. After treatment with 0.5 N NaOH to dissolve the products of grinding, the particle size decreased with increased grinding time for the shorter periods of grinding and then increased. The treatment was sufficient to dissolve most of the products of grinding from those samples ground only a short time but not from those ground for longer periods. Comparison of figure 4A with 3A, 4B with 3B, and so forth, shows the effect of the NaOH. The treatment had little effect on the unground sample and on the sample ground for 72 hours it dissolved nearly all the products of grinding, leaving crystal fragments with sharp edges. In the two other cases there was still a considerable amount of amorphous material coating the particles, showing that treatment with sodium hydroxide under the conditions of the experiment was not sufficient to remove all the products of grinding from these samples. Thus it can be seen that the change in effective particle size, due to grinding, is additional evidence for the formation of a permutite-like material.

TABLE 4

*Effects of grinding kaolinite upon the water loss (weight loss) at various temperatures*

GRINDING TIME hrs.	PERCENTAGE OF SAMPLES LOST			
	to 150°C.	150°-400°C.	400°-800°C.	150°-800°C.
0	1.1	1.9	11.5	13.4
24	3.7	3.2	10.7	13.9
48	5.4	5.0	8.9	13.9
72	6.6	5.1	8.0	13.1
96	7.5	5.9	7.0	12.9
144	8.2	6.4	6.3	12.7

#### *Dehydration and differential analysis*

In their work on the dehydration of minerals, Kelley, Jenny, and Brown (7) have shown that grinding clay minerals increased the total moisture coming off below 400°C. and that this increase was due to water adsorbed from the atmosphere and to a decrease in the constitutional water which is driven off by heating the mineral above 400°C. They defined "adsorbed water" as that portion of the total water which comes off at 400°C., with the condition that there is no evidence that true crystal lattice water is included in this category. They divided the "adsorbed water" into two classes: *planar water* which is weakly held on the surfaces of the crystals and readily lost at 150°C.; and *broken-bond water* held on the edges of crystals or crystal fragments by a free "bond" of Al, Si, or O. This *broken-bond water* is held by much stronger forces than the *planar water* and was considered as being given off between 150° and 400°C.

Table 4 shows that there was an apparent decrease in constitutional water with each increase in grinding time. The explanation given by Kelley, Jenny, and Brown (7) for the decrease in constitutional water due to grinding of the mineral was that the OH ions in the interior of the crystal are surrounded by other atoms and ions which make it difficult for water to escape, but if these OH ions are brought to the surface by grinding they are held by the particle on one

side only, thus they experience more freedom of movement, the extent of thermal agitation is greater, and they escape at a lower temperature. In other words, the superficial crystal lattice OH ions act as adsorbed water, presumably as broken-bond water.

Figure 6 is a schematic presentation of the crystal structure of kaolinite showing a surface OH at A and six free "bonds." These free bonds would attract

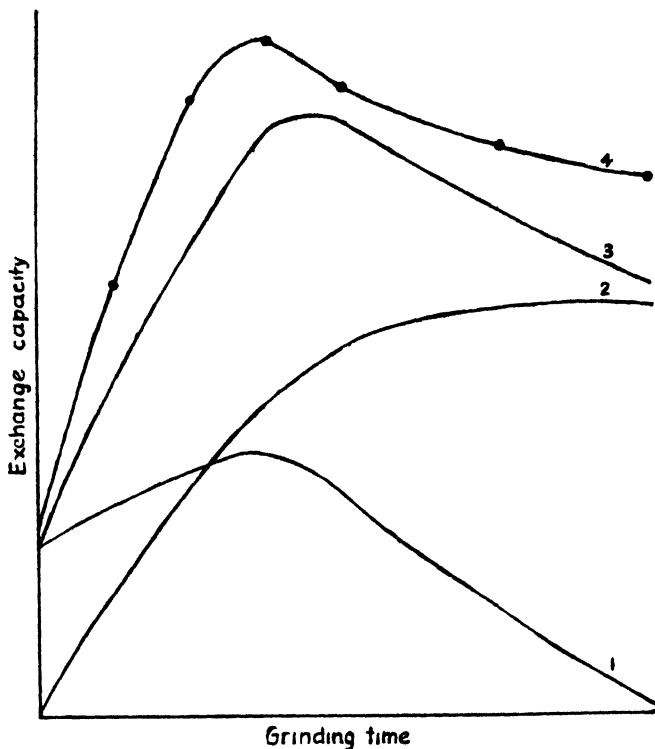


FIG. 5. FACTORS CONTRIBUTING TO THE EFFECTS OF GRINDING ON EXCHANGE CAPACITY

Hypothetical curves for 1, contribution of broken edges; 2, amorphous material; 3, total effect (sum of 1 and 2). Curve 4, actual exchange capacity (fig. 2A, curve 1).

and orient water molecules from the surrounding atmosphere which should appear on dehydration as *broken-bond water*. Presumably the OH ion at A would appear in the same category, since it is held by what might be considered as a free Al bond.

If the crystal is fractured at the dotted line there immediately come into play one more surface OH at B and five more free "bonds." If the OH at A and B acted as *broken-bond water* and were lost below 400°C., the constitutional water would be decreased slightly but the *broken-bond water* would be increased considerably because, presumably, at least three OH ions and two H ions would be adsorbed from the atmosphere by the five additional free "bonds." This, plus the OH ion at B, would give three molecules of  $\text{H}_2\text{O}$ . It seems clear, then, that

the sum of *broken-bond water* and lattice water should increase with increased grinding time. The data in table 4 show that such was not the case. In fact, there was a slight decrease in this sum. While the loss of superficial OH ions from kaolinite crystal fragments may not account for the marked decrease in lattice water, it would most assuredly account for the slight decrease in the sum of the lattice water and *broken-bond water*.

Differential thermal analysis curves were made of ground samples having had no previous treatment. The apparatus used was the reflecting galvanometer type which records the deflections on a photographic plate. One galvanometer records the temperature of the nickel block in which the sample is held, the other, the temperature difference between the sample and the reference material (calcined alumina). The heating rate was 15°C. per minute. The samples were

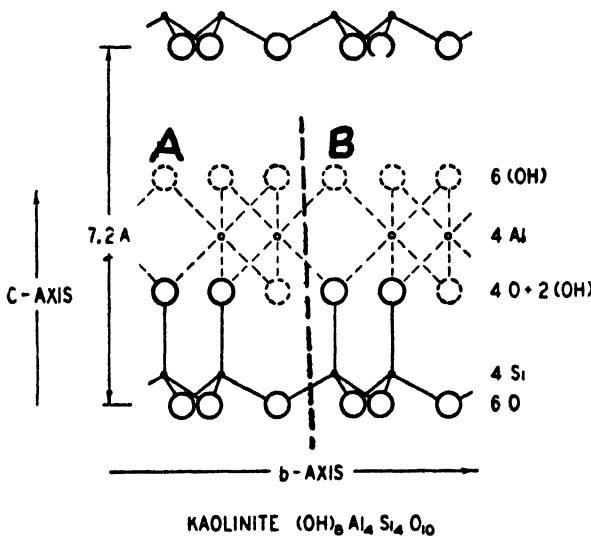


FIG. 6. SCHEMATIC PRESENTATION OF THE CRYSTAL STRUCTURE OF KAOLINITE, SHOWING INCREASE IN FREE BONDS DUE TO FRACTURE OF THE CRYSTAL

After Gruner (3)

kept over a sulfuric acid-water mixture of 80 per cent relative humidity for a month before being run.

Figure 7 shows tracings of curves obtained for the series of samples studied. Each of the ground samples shows an endothermic reaction below 350°C. which increases in depth with increased grinding time. This is in keeping with the suggestion that grinding produces a new compound that is unstable below 400°C. The endothermic reaction representing loss of lattice water came at approximately 500°C. except in the sample ground 192 hours, where it was about 50°C. lower. The exothermic reaction occurs between 920 and 980.

The area under the curve is a measure of the amount of water lost over a given temperature range. The area under the curve representing the loss of lattice

water was determined by tracing the curves on cross-section paper and counting the squares. By taking the area under the curve of the unground sample as representative of the water lost by that sample between 400 and 600° when dried

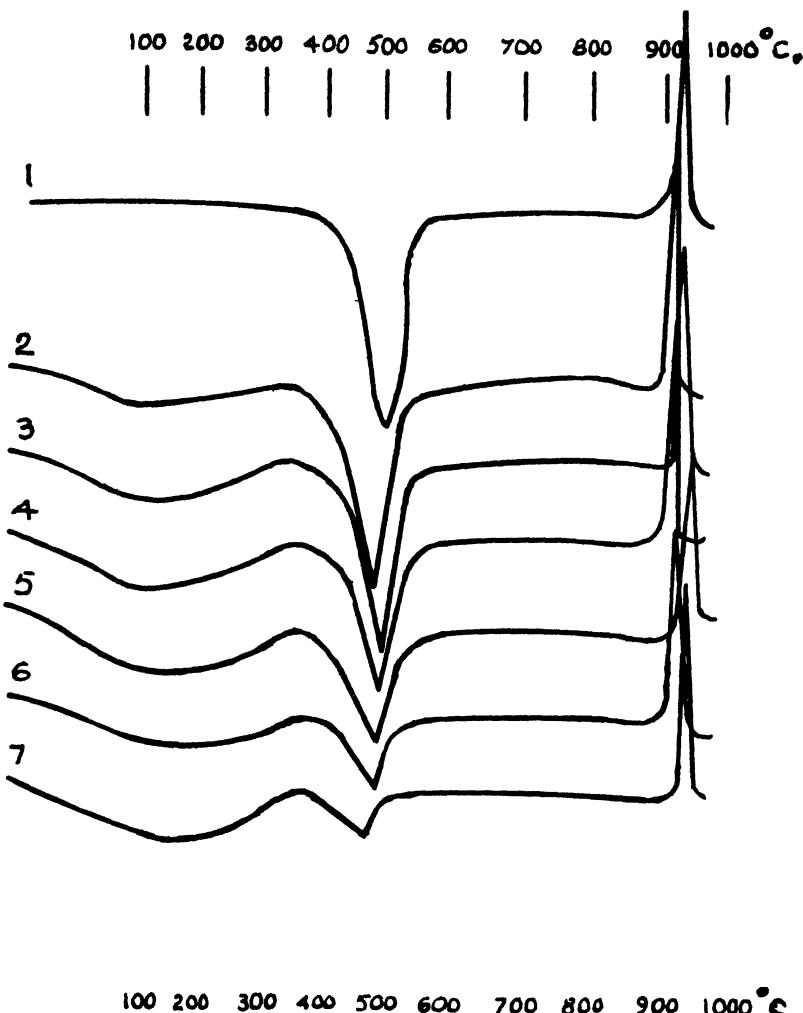


FIG. 7. DIFFERENTIAL THERMAL ANALYSIS CURVES OF GROUND KAOLINITE SAMPLES  
1, Unground; 2, ground 24 hours; 3, ground 48 hours; 4, ground 72 hours; 5, ground 96 hours;  
6, ground 144 hours; and 7, ground 192 hours

in the oven, the percentage of true lattice water lost by each sample was calculated. The area of each curve was measured from the point where the curve breaks downward to the point where it levels off again. In table 5 the percentage of water lost between these two temperatures is compared with that lost over the same temperature range when some of the same sample was dried in the oven and weighed.

*Action of 0.5 N sodium hydroxide*

As mentioned previously, an effort was made to free the samples of the permutite-like material produced by grinding so as to leave only the unchanged kaolinite. To do this, the samples were digest in 0.5 N sodium hydroxide for 3 days at 65° C. The sodium hydroxide and washings were analyzed for silica and alumina, and the exchange capacity of the undissolved material was determined by the ammonium acetate method. Later, additional samples were treated similarly with 0.5 N sodium hydroxide followed by washing with 0.1 N hydrochloric acid before the analysis for silica and alumina and the determination of the exchange capacity. The data are presented in tables 3 and 6.

The data in table 6 show that the silica soluble in 0.5 N sodium hydroxide increased with grinding time up to the 48-hour period and then continued to decrease. The alumina dissolved increased up to 48 hours and remained fairly

TABLE 5

*Comparison of data obtained from differential thermal curves with those obtained from drying*

GRINDING TIME	LATTICE WATER LOST	
	I*	II†
hrs.	per cent	per cent
0	10.5‡	10.5‡
24	10.2	10.7
48	9.3	9.7
72	7.7	7.6
96	7.7	7.1
144	3.9	4.7
192	1.9	...

\* Values obtained from area of differential thermal curves

† Values from oven-drying curves covering same temperature range.

‡ Value used to calibrate area of curve 1, figure 7

constant, declining only slightly. The explanation for this phenomena is not clear.

After the exchange capacity had been determined, the samples were treated with sufficient hydrochloric acid to remove the magnesium oxide added during the determination of the exchange capacity. Electron micrographs and electron diffraction patterns were taken after this final treatment. In figure 8 the micrographs and diffractions patterns, before any "cleaning" treatment and after this final treatment are compared for kaolinite ground 192 hours. The sharp lines in the figure and the data in table 7 show that the treatments with 0.5 N NaOH and 0.1 N HCl plus distillation with magnesium oxide and treatment with hydrochloric acid removed the amorphous material leaving only crystal fragments with definite structure. This is rather conclusive evidence that the crystal fragments remaining are kaolinite, and that the entire lattice structure of the original crystals has not been distorted, as was suggested by Marshall (10).

It is interesting to compare the results obtained and the explanations given with the work of other investigators. Trenel and Wenschik (15) found that a permutite decomposes into its constituents with progressive removal of bases,

TABLE 6  
*Solubility of silica and alumina from kaolinite as affected by grinding*

GRINDING TIME	0.5 N NaOH TREATMENT		0.5 N NaOH AND 0.1 N HCl TREATMENT	
	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>
hrs.	per cent*	per cent*	per cent*	per cent*
0	3.17	5.75	3.49	6.70
24	3.63	8.73	4.40	10.29
48	4.02	10.35	7.87	12.14
72	3.60	10.57	9.13	15.20
96	2.73	10.75	13.53	21.91
144	1.73	10.38	16.36	29.35
192	1.42	9.54	21.13	

\* Percentages of original sample used.

TABLE 7  
*Electron diffraction data for unground kaolinite and kaolinite ground 192 hours and thoroughly "cleansed"*

UNGROUND		GROUND 192 HOURS	
Intensity*	d	Intensity	d
	A.		A.
vs	4.47	vs	4.46
s	2.59	s	2.57
w	2.53	w	2.50
		vw	2.34
vw	2.24	vw	2.24
m	1.69	m	1.69
vs	1.49	s	1.49
m	1.29	w	1.29
m	1.24	m	1.24
w	1.120	w	1.117
w	1.025	w	1.032
w	0.975	vw	0.976
vw	0.896		
w	0.861		
w	0.844		
vw	0.802		

\* s, strong; w, weak; m, moderate; vs, very strong; vw, very weak.

and that 0.1 N organic acids completely decompose the permutite leaving a residue which is apparently composed of SiO<sub>2</sub> and shows only traces of "exchange acidity." This is in agreement with the results obtained in this work when the samples treated with 0.5 N NaOH received additional acid washings. The fact

that washing with 0.1 *N* HCl removed large amounts of additional alumina and silica from the NaOH-treated samples of ground kaolinite seems to correlate with Stout's (14) findings that dephosphated kaolinite did not give an x-ray pattern until it was washed with 0.001 *N* HCl.

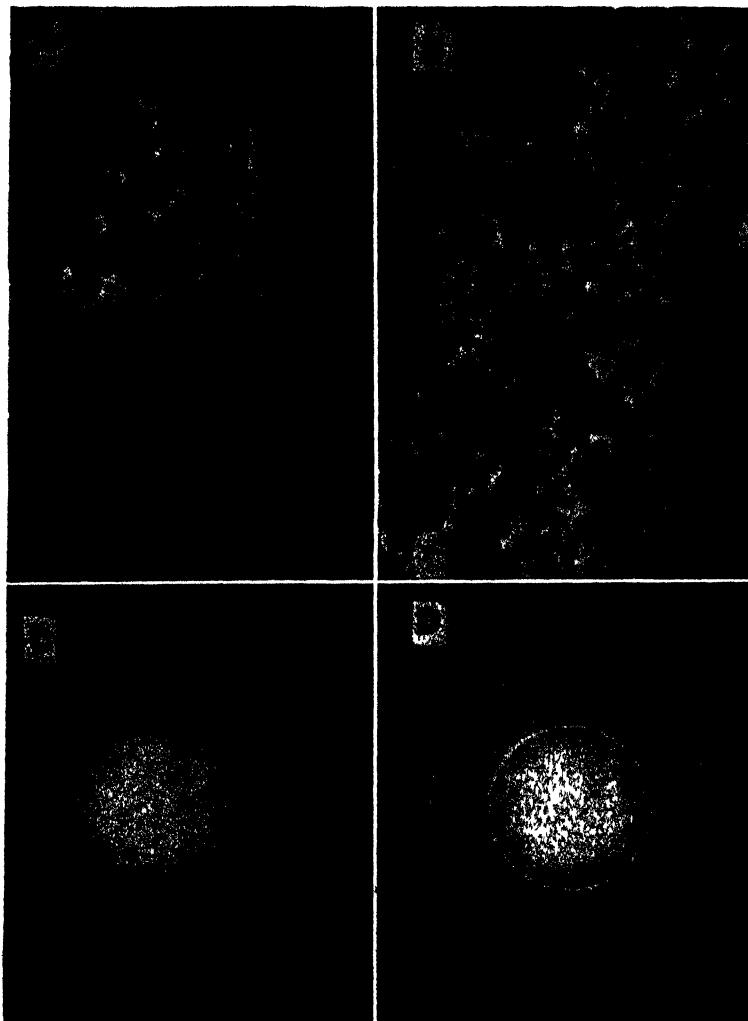


FIG. 8. KAOLINITE GROUND 192 HOURS. A, SELECTED AREA OF FIELD FOR PATTERN C (UNTREATED); B, SELECTED AREA FOR PATTERN D (FREED FROM AMORPHOUS MATERIAL)  
\* Line in A and B represents 1  $\mu$

The suggestion that digestion of the ground samples in 0.5 *N* sodium hydroxide resulted in the formation of a permutite is in agreement with the work of Delimarkii *et al.* (1) who prepared permutites by digesting finely ground kaolinite for 3 hours with NaOH in a porcelain crucible on a steam bath. Thirty per cent NaOH gave the best results, but other concentrations produced permutites.

In addition to the investigations reported in this paper, a parallel study, which included only a 192-hour grinding period, was made on a sample of kaolinite originally much coarser than the one used throughout this investigation. The results from the two samples were very similar. The data for this additional sample were not included, since they largely repeat those presented.

#### SUMMARY AND CONCLUSIONS

Dry grinding of kaolinite for long periods of time results in the formation of a new permutite-like material designated a precursor of permutite because:

The exchangeable bases on the original sample decreased with increased grinding time.

The pH of the ground samples was not appreciably altered (bases were not removed) by electrodialysis.

More calcium was fixed by the ground than by the unground samples when leached with neutral  $\text{CaCl}_2$ .

The effective particle size and exchange capacity increased with grinding up to 96 hours and then decreased.

After long periods of grinding, the exchange capacity curves tend to level off at a higher level than the exchange capacity of the unground kaolinite. This should not happen if fine grinding resulted in a simple mixture of silica and alumina.

Treatment of the ground kaolinite with 0.5 N NaOH resulted in very high exchange capacities for the ground samples. The formation of a permutite was suggested as the explanation of the high exchange capacities found.

Treatment with 0.1 N HCl following the NaOH treatment destroys the suggested permutite and results in a very marked lowering of the exchange capacity.

After all the amorphous material had been removed from the ground samples, the crystal fragment left gave a sharp electron diffraction identical with unground kaolinite.

#### REFERENCES

- (1) DELIMARKII, Y., ZINKOVA, Z., AND GOLDMAN, G. 1936 The preparation of permutites from kaolinite. *Univ. Kiev, Bul. Sci. Rec. Chem.* 2 (2): 29-34. (Original not seen. Abstract in *Chem. Abs.* 31: 3218, 1937.)
- (2) EITEL, W. VON, MULLER, H. O., AND RADIZEWSKI, O. E. 1939 Uebermikroskopische Untersuchungen an Tonmineralier. *Ber. Deut. Ker. Gesell.* 20: 169-179.
- (3) GRUNER, J. W. 1932 The crystal structure of kaolinite. *Ztschr. Krist.* 85: 75-88.
- (4) JACKSON, M. L., AND TRUOG, E. 1939 Influence of grinding soil minerals to near molecular size on their solubility and base-exchange properties. *Soil Sci. Soc. Amer. Proc.* 4: 136-143.
- (5) KELLEY, W. P., DORE, W. H., AND BROWN, S. M. 1931 The nature of base-exchange material of bentonite, soils and zeolites as revealed by chemical investigations and x-rays. *Soil Sci.* 31: 25-55.
- (6) KELLEY, W. P. 1936 The evidence as to the crystallinity of soil colloids. *Trans. 3rd Internat. Cong. Soil Sci.* 3: 88-91.
- (7) KELLEY, W. P., JENNY, H., AND BROWN, S. M. 1936 Hydration of minerals and soil colloids in relation to crystal structure. *Soil Sci.* 41: 259-274.
- (8) KELLEY, W. P., AND JENNY, H. 1936 The relation of crystal structure to base exchange and its bearing on base exchange in soils. *Soil Sci.* 41: 367-382.
- (9) KELLEY, W. P. 1939 Base exchange in relation to sediments. *Amer. Assoc. Petroleum Geol. Recent Marine Sediments*, Sept. 1939: 454-465.

- (10) MARSHALL, C. E. 1937 The colloidal properties of clays as related to their crystal structure. *Jour. Phys. Chem.* 41: 935-942.
- (11) PAGE, J. B., AND BAVER, L. D. 1939 Ionic size in relation to fixation of cations by colloidal clay. *Soil Sci. Soc. Amer. Proc.* 4: 150-155.
- (12) SHAW, B. T., AND HUMBERT, R. P. 1941 Electron micrographs of clay minerals. *Soil Sci. Soc. Amer. Proc.* 6: 146-149.
- (13) SHAW, B. T. 1942 The nature of colloidal clay as revealed by the electron microscope. *Jour. Phys. Chem.* 46: 1032-1043.
- (14) STOUT, P. R. 1939 Alterations in the crystal structure of clay minerals as a result of phosphate fixation. *Soil Sci. Soc. Amer. Proc.* 4: 177-182.
- (15) TRENEL, M., AND WENSCHIK, J. 1930 The chemistry of the "mineral soil acidity" *Ztschr. Pflanzenernähr., Düngung, Bodenk.* 17A: 257-296. [Original not seen. Abstract in *Chem. Abs.* 24: 5911.]
- (16) VANSELOW, P. A. 1932 Equilibria of the base-exchange reactions of bentonites, permutites, soil colloids, and zeolites. *Soil Sci.* 33: 95-113.
- (17) WIEGNER, G., AND JENNY, H. 1927 Basen-austausch an Permutiten. *Kolloid Ztschr.* 42: 268-272.

# BORON SUPPLY AND BORON CONTENT OF POTATOES<sup>1</sup>

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Previous investigation (5) of the effect of cultural conditions on the boron content of actively functioning leaves and mature tubers of potatoes grown in Wisconsin indicated a rather wide and consistent variation from location to location. The purpose of the present study was to attempt to correlate these variations with the supply of "available" boron in the soil and to study the effect of boron fertilization on the content of this element in the tissues of the plant.

## MATERIALS AND METHODS

Leaf and tuber tissues were obtained during the season of 1941 from the adaptation trial plots of the University's potato improvement program at Almond on Waukesha sandy loam, at Coddington on an unclassified peat soil, at Antigo on Antigo silt loam, and at Three Lakes on Vilas sandy loam. Seed was obtained from disease-free foundation stock produced at the Wisconsin State Seed Farm at Three Lakes. Standard commercial growing practices were followed. No boron was supplied in the fertilizer treatment.

Leaf and soil sampling was done on July 23, 1941. Care was taken to select leaves of the same degree of maturity at each location, the fifth or sixth basal leaf being selected. The leaf blades were detached from the stem and petioles on July 24, dried at 60° F. in a current of air, ground to pass a 40-mesh sieve, and stored for later analysis. Tuber tissue samples were taken at the time of removal of the tubers from storage in January, 1942. Twenty representative tubers were selected and thoroughly washed, rinsed in distilled water, and dried. Each tuber was then bisected longitudinally, and one half was cut into thin slices by a slicing machine. The slices were rapidly heated in an oven to 90° C. and then dried to a constant weight in a continuous stream of air at 60° C. The dry material was then ground to pass an 80-mesh sieve and stored. Soil samples were taken in quadruplicate from each plot, pooled, and thoroughly mixed. An aliquot was then taken for analysis.

Studies on the effect of boron fertilization were made on experiment plots of the University on Miami silt loam near Madison. Chippewa variety certified stock produced at Antigo was planted on May 5, 1941 in plots 8 by 40 feet. Hills were placed 2 feet apart. There were six buffer hills between plots; each treatment

<sup>1</sup> Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

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was replicated four times. Boron was applied in the form of borax at the rate of 25, 50, 100, and 150 pounds per acre. It was placed in two bands approximately 6 inches from the center of the row and was worked into the soil. On July 24, 1941 and September 15, 1941 respectively, leaf and tuber samples were taken in the manner previously described.

TABLE 1

*Relation of soil type and soluble soil boron to the ash and boron contents\* of actively functioning leaves and mature tubers of potatoes*

LOCATION AND VARIETY	YIELD tons/A.	YIELD lbs./hill	SOLUBLE BORON IN SOIL p.p.m.	LEAF TISSUE		TUBER TISSUE	
				Ash per cent	Boron γ/gm.	Ash per cent	Boron γ/gm.
Waukesha sandy loam, Almond							
Triumph.....	2.63	1.27		11.2	45.4	7.2	9.5
Cobbler.....	3.65	1.76		11.9	38.5	6.2	10.4
Chippewa.....	3.80	1.85		11.9	47.1	6.8	13.3
Rural New Yorker .....	3.86	1.90		12.2	49.9	6.5	9.8
Average.....	3.49	1.69	0.54	11.8	45.2	6.7	10.7
Unclassified peat, Coddington							
Triumph.....	11.70	2.02		9.2	32.2	5.6	7.7
Cobbler.....	11.23	1.93		10.2	30.5	4.6	7.1
Chippewa.....	12.41	2.15		10.6	31.0	6.2	9.2
Rural New Yorker .....	11.65	2.00		10.2	32.7	4.8	7.2
Average.....	11.77	2.02	2.10	10.0	31.6	5.3	7.8
Antigo silt loam, Antigo							
Triumph.....	7.41	1.04		9.0	42.5	4.6	8.2
Cobbler.....	6.48	0.93		10.3	33.5	4.4	9.5
Chippewa.....	6.65	0.97		11.2	34.8	4.1	14.3
Rural New Yorker .....	7.87	1.25		10.0	40.3	4.6	7.7
Average.....	7.10	1.05	0.73	10.1	37.8	4.4	9.9
Vilas sandy loam, Three Lakes							
Triumph.....	8.98	1.44		9.0	26.1	5.5	9.1
Cobbler.....	9.50	1.53		10.3	22.0	4.4	6.4
Chippewa.....	10.15	1.64		11.2	24.0	4.8	8.2
Rural New Yorker .....	9.55	1.54		10.0	25.3	4.9	7.4
Average.....	9.55	1.54	0.36	10.4	24.4	4.9	7.8

\* Ash and boron contents on a moisture-free basis.

**Ash.** Ash was determined on the air-dry samples by incineration in a thermostatically controlled muffle furnace at 800° C.

**Boron.** The quinalizarin procedure (1) as modified and adapted to the photoelectric colorimeter (5) was used for the determination of boron. The hot water soluble boron was determined as outlined by Berger and Truog (3). This fraction has been used by them (2) and by DeTurk and Olson (4) as a measure of the available boron of the soil.

## RESULTS

Plants on the adaptation trial plots at all locations had made vigorous growth by the time of tissue sampling. There were no signs of boron deficit although no boron had been included in the fertilizer treatment.

Tuber yields, soluble boron content of soils, and total boron content of leaves and tubers are presented in table 1.

Examination of the ash and boron contents of leaves and tubers of the four varieties showed no outstanding differences. This is in accordance with previous findings (5). The boron content of leaves and, in most instances, of tubers increased progressively in the following order of locality: Three Lakes, Coddington, Antigo, Almond. These variations may be correlated with the soluble boron content only on soils similar in texture. Thus, the higher boron content of leaves and tubers grown on Waukesha sandy loam as compared with Vilas sandy loam

TABLE 2

*Effect of boron fertilization on the yield of tubers and on the boron and ash contents\* of actively functioning leaves and mature tubers of potatoes grown on Miami silt loam*

TREATMENT	YIELD OF TUBERS PER HILL	SOLUBLE BORON IN SOIL	LEAF TISSUE		TUBER TISSUE	
			Ash	Boron	Ash	Boron
No fertilization.....	2.6	1.0	12.2	41.5	5.7	11.2
25 lbs. Borax/acre.....	2.6	1.8	11.7	95.8	6.1	34.2
50 lbs. Borax/acre.....	2.5	2.1	11.6	139.0	5.5	55.0
100 lbs. Borax/acre.....	1.8	5.6	12.0	315.0	5.6	94.4
150 lbs. Borax/acre.....	1.2	11.1	12.4	450.0	6.3	121.0

\* Ash and boron contents on a moisture-free basis.

was associated with more soluble boron on the former soil. Less boron was absorbed by plants on the heavier soil at Antigo and the peat at Coddington than at Almond although both contained larger amounts of soluble boron; the lower density of the soil at Almond, however, must be considered. It seems unlikely that the pH of the soil is responsible for this inconsistency, since the soils were all distinctly acid, 4.3, 5.2, 4.5, and 5.0 at Three Lakes, Antigo, Coddington, and Almond respectively.

Plants grown under varying levels of boron fertilization in Madison made excellent growth in those plots receiving no borax or 25 pounds per acre. Plants in plots receiving 50 pounds showed injury to the foliage during the first 6 weeks of growth as evidenced by a marginal necrosis of the mature leaves. This condition, however, did not persist, and at the end of the growing season these plants were essentially normal in external appearance. The application of 100 and 150 pounds of borax per acre resulted in permanent injury. As the growing season progressed, the newer leaves of plants receiving these levels became thickened and malformed, while the older leaves exhibited continued marginal necrosis. Such plants were also reduced in total vegetative and tuber growth. Yields of

tubers, soluble soil boron at the end of the growing season, and total boron and ash contents of leaf and tuber tissue are presented in table 2.

The data show a correlation of the boron content of the leaves and tubers, the quantity of borax applied, and the quantity of soluble boron in the soil at the end of the growing season. The boron content of leaves and tubers grown on plots supplied with 150 pounds of borax per acre was approximately eleven times that of plants receiving no additional boron. The soluble soil boron was also approximately eleven times that found in unfertilized plots. The data also reveal evidence of toxicity at levels above 50 pounds per acre. It would appear that the potato plant can tolerate boron levels of 100 to 150 p.p.m. in leaf tissue, and that levels in excess of 300 p.p.m. are definitely toxic. These higher levels are associated with soluble boron levels in excess of 5 p.p.m. in this particular silt loam soil.

It would appear from these findings that the interpretation of soluble boron analyses in soils should be confined to soils of similar texture unless accompanied by analysis of plant tissues produced on the soils in question.

#### SUMMARY AND CONCLUSIONS

The boron content of potato leaf and tuber tissue can be correlated with soluble soil boron on soils of similar texture.

In experimental plots receiving borax fertilization, toxicity was observed when the soluble soil boron exceeded 5 p.p.m. in Miami silt loam or the total boron exceeded 300 and 100 p.p.m. in leaf and tuber tissues respectively.

#### REFERENCES

- (1) BERGER, K. C., AND TRUOG, E. 1939 Boron determination in soils and plants. *Indus. and Engin. Chem., Analyt. Ed.* 11: 540-545.
- (2) BERGER, K. C., AND TRUOG, E. 1940 Boron deficiencies as revealed by plant and soil tests. *Jour. Amer. Soc. Agron.* 32: 297-301.
- (3) BERGER, K. C., AND TRUOG, E. 1944 Boron tests and determination for soils and plants. *Soil Sci.* 57: 25-36.
- (4) DETURK, E. E., AND OLSON, L. C. 1941 Determination of boron in some soils of Illinois and Georgia. *Soil Sci.* 52: 351-357.
- (5) MACVICAR, R., TOTTINGHAM, W. E., AND RIEMAN, G. H. 1941 The comparative boron content of potato leaves and tubers produced under different cultural conditions. *Amer. Potato Jour.* 18: 249-253.

## BOOKS

*Anaerobic Corrosion of Iron in Soil.* By R. L. STARKEY AND K. M. WIGHT. American Gas Association, New York, 1945. Pp. 108, figs. 27.

This is the final report on the American Gas Association iron corrosion research fellowship. It gives a complete review of the literature on the subject, including a bibliography of 203 publications. The report covers detailed studies of specific bacteria that cause corrosion under conditions where there is no free oxygen, with the resulting production of ferrous hydrate and ferrous sulfide. Since the process is biological, it is affected by the moisture content of the soil, the soil reaction, its content and type of organic matter, and the supply of nutrients. The methods employed are applicable for wide use in the study of drainage and aeration problems in soils and should be of great interest to workers in that field.

*Electronic Equipment and Accessories.* By R. C. WALKER. Chemical Publishing Co., Inc., Brooklyn, New York, 1946. Pp. 393, figs. 343. Price \$6.

The purpose of this book is to present the subject of electronics and its applications in the field of engineering. The several chapters deal with thermionic valves, and their application, gas-filled valves and their application, light-sensitive devices and methods of their use, sources of light, cathode-ray tubes and their use, miscellaneous electronic devices, small switch gear, delayed action devices, impulse recording, and miscellaneous circuit accessories. The book will be of special value to the more practical-minded electrical engineer and mechanic who will find it very useful for reference purposes.

*Forests Soils and Forest Growth.* By S. A. WILDE. The Chronica Botanica Co., Waltham, Massachusetts; G. E. Stechert and Co., New York, 1946. Pp. 241, figs. 38, plates 7. Price \$5.

This is the eighteenth volume of a series of plant science books published by the Company. It is an interesting book. It deals with the history of the study of forest soils, genesis of forest soils, genetic soil groups of the world, forest cover, physical, chemical, and biological properties of forest soils, forest humus, soil-forest types, forest soil survey, soils and tree planting, amelioration of forest soils, silvicultural cuttings in relation to soils, forest management, forest nurseries, use of composts and green manures, nursery fertility, and control of parasitic organisms in soils of nurseries. The author has made a highly important contribution not only to the literature of this field of study but to the general subject of soil-plant relationships.

*German for the Scientist.* First American Edition. By PETER F. WIENER. Chemical Publishing Co., Inc., Brooklyn, New York, 1946. Pp. 238. Price \$3.50.

This book is designed for those who are trying to improve their reading capacity for German in the shortest possible time. The first part deals with the elements

of German grammar, the second and third contain representative selections from articles on chemistry and physics, respectively, and the fourth gives the English translations for the material contained in parts two and three. The book should be especially useful to graduate students with little or no training in classical German.

*Introduction to Atomic Physics.* Revised and Enlarged. By HENRY SEMAT. Rinehart & Company, Inc., New York, 1946. Pp. 412, figs. 168. Price \$4.50.

This book presents the subject of atomic physics in an especially clear and attractive manner. It is divided into nine chapters which deal with electricity and magnetism, elementary charged particles, the hydrogen atom, atomic spectra and electron distribution, natural radioactivity, disintegration of nuclei, and nuclear energy. A total of 144 topics are considered. The appendix consists of nine very useful tables and sets of equations. Transuranic elements, the fission of nuclei, and nuclear isomers have been given more extensive treatment than in the first edition. The new material includes new elements, sources of nuclear energy, and the measurement of magnetic moments. This book should be very useful both to those who are working in this field and to those who desire to be brought up to date on recent developments in it.

*Quantitative Analysis with Applications to Agricultural and Food Products.* By HOWARD O. TRIEBOLD. D. Van Nostrand Company, Inc., New York, 1946. Pp. 331, figs. 47. Price \$3.

This book is designed for use in teaching quantitative chemistry to students in a college of agriculture, illustrative determinations being chosen from that field of study. The book is made up of seven parts: fundamentals, gravimetric analysis, volumetric analysis, physical chemical methods, general methods in agricultural and food analysis, specific methods for agricultural products, and stoichiometry. The agricultural products include feedstuffs, milk, butter, maple syrup, soils, fertilizers and limestone, and insecticides and fungicides. The methods are well chosen and the material is well presented. A very useful set of 135 problems is appended. The book should find an important place in the literature in this field.

*Vapor Adsorption.* By EDWARD LEDOUX. Chemical Publishing Co., Inc., Brooklyn, New York, 1945. Pp. 360, figs. 144. Price \$8.50.

This book is designed to give the reader a comprehensive view of our present concepts of the mechanism of adsorption, the uses to which it may be put, and the equipment employed in its use. It is divided into four parts, the first of which deals with static adsorption, the second with vapor and heat transfer and air saturation as related to the application of adsorption to industrial processes, the third with dynamic adsorption, and the fourth with some of the more outstanding industrial problems for which adsorption methods provided the answer. Those who are concerned with soil-plant science will find a variety of useful applications for the principles presented in this volume.

THE EDITORS.

# THE "RHIZOSPHERE EFFECT" OF MANGELS ON CERTAIN GROUPS OF SOIL MICROORGANISMS<sup>1</sup>

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The selective action of plant roots on different groups of soil bacteria has been studied by many investigators. Fungi and actinomyces have received less attention, and protozoa and algae virtually none, yet these four groups of organisms constitute a very important fraction of the soil population. The emphasis on bacterial groups or species has also been disproportionate. Organisms such as *Azotobacter*, *Bacterium radiobacter*, and aerobic spore-formers, have been studied to the exclusion of other forms such as cellulose-decomposing bacteria and particularly anaerobes (nitrogen-fixers and others) despite the fact that these latter groups are abundant in soil and under certain conditions very active in soil transformations. Some results of studies on the prevalence of these and other groups of microorganisms in the rhizosphere of mangels as influenced by age of plant and soil treatment are presented in this paper.

## EXPERIMENTAL

Samples of soil and root were collected at three stages of growth of the plant for determination of the abundance of certain groups of organisms and at weekly intervals throughout the growing season for others. The first samples were taken 80 days after seeding when the plants were still small but reflecting clearly, even at this stage, the difference in treatment of the two soils used, N—receiving no fertilizer, and X—receiving farmyard manure (15 tons per acre) continuously for 30 years and consequently much more productive than soil N (9). In soil N, the roots were commonly no more than  $\frac{1}{4}$  to  $\frac{1}{2}$  inch in diameter at the crown, and poorly developed, whereas soil X supported well-rooted (1 inch in diameter), vigorously growing plants.

Samples of soil were taken at a depth of 2 to 6 inches, midway between rows. Roots were shaken to dislodge adhering soil clumps, and samples were placed in sterile water in weighed flasks, which were then shaken vigorously for 5 minutes. Dilutions were made and aliquots removed for analysis. The root segments were then transferred to a funnel and washed. All wash water and dilutions with soil sediment were composited and returned gradually to the original weighed flasks and evaporated in an oven at 105°C. Thus all the soil in the rhizosphere sample except that used for the actual analytical manipulations was collected and weighed.

Plate counts were made in quadruplicate, dilution series in duplicate, and

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all calculations were based on oven-dry soil. The following determinations were made:

1. Bacteria and actinomyces developing upon soil extract agar (10, 18); counted after 12 days at 25°C.
2. Nitrifying bacteria developing in ammonium sulfate solution (2, medium 48). Tests for ammonia and nitrites were made after 10, 20, and 30 days at 25°C.
3. Ammonifying bacteria growing in tubes in a 1 per cent gelatin medium containing inorganic salts (2, p. 128); tests for ammonia were made after 6 and 12 days at 25°C.
4. Denitrifying bacteria growing in an asparagine nitrate solution in tubes (2, medium 55); tests for nitrates, nitrites, and ammonia were made after 6 and 12 days at 25°C.
5. Fungi developing on Smith and Dawson's soil-extract-glucose-nitrate agar medium (11) containing rose bengal (dilution 1:15,000) to inhibit bacterial growth; counted after 3 and 5 days at 25°C.
6. Algae developing in Bristol's sodium nitrate solution in tubes (2, medium 43, procedure p. 97).
7. Protozoa; 1 ml. of each dilution was spread uniformly on nutrient agar plates. Wet mounts were made at 7 and 14 days; plates were kept at room temperature and moistened when necessary with sterile water.
8. *Azotobacter*; 1 ml. of each dilution was spread uniformly on nitrogen-free-mannite agar plates [Lipman's medium (19) with added Mn and Mo]; examined macroscopically and microscopically after 3 and 7 days at 25°C.
9. Cellulose-decomposing bacteria growing in Omeliansky's medium (2, medium 85, procedure p. 134); examined at 7 and 14 days.
10. Anaerobic bacteria growing in deep peptone-glucose agar tubes (2, medium 12, procedure p. 100).

#### EXPERIMENTAL RESULTS

The results presented in figures 1 and 2 reveal the striking effect exerted by mangel roots in both soils on numbers of bacteria, actinomyces, fungi, ammonifying and denitrifying bacteria, and protozoa. The "rhizosphere effect" on algae was most evident in the latter part of the growing season; on nitrifiers, it was most evident during the earlier period, though even at that time, it was not very pronounced. With the exception of these two groups, numbers of organisms appeared to be at a maximum in the rhizosphere of plants in the manured soil X at the second sampling period, after which a general decline usually occurred. The selective action of the roots in this soil at 117 days may be expected in view of the vigorous growth occurring at this time. Rhizosphere : soil ratios, obtained by dividing the number of organisms in the rhizosphere by the number in the soil at a distance from the root, show the same trends (table 1), a fact which indicates that the observed increases at this period are due to the influence of the actively functioning roots themselves.

The selective action of the roots in soil N on most of these groups of organisms begins to manifest itself within 142 days, at a time when the much more slowly growing plants in this unfertilized soil are reaching an appreciable size, somewhat comparable to that of plants in manured soil at 117 days. The importance of considering rhizosphere : soil ratios of the counts obtained rather than counts alone in order to demonstrate an actual "rhizosphere effect" should be noted at this point. For example, it may be seen in figure 1 that counts of actinomyces

in the rhizosphere of plants in soil N reach a peak at 117 days and decrease at 142 days, carrying the implication that the "rhizosphere effect" is diminished at the latter period. Examination of table 1 shows, however, that the selective action of the roots is even more powerful at 142 days than at 117, the rhizosphere : soil ratios being 30 and 23 respectively. Environmental factors have apparently caused a general decrease of these organisms, but the "rhizosphere effect" is still very pronounced. The ratios are also of great importance in comparing the effect of roots in the two soils. Figure 2 shows

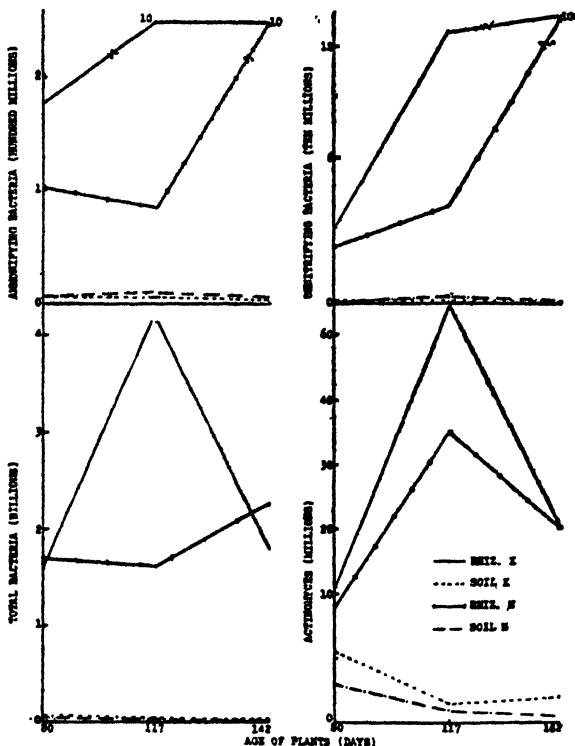


FIG. 1. INFLUENCE OF SOIL TREATMENT AND AGE OF PLANT ON MICROBIAL NUMBERS IN THE RHIZOSPHERE OF MANGELS

that at 142 days counts of algae were considerably higher in the rhizosphere of plants in soil X than in soil N. The rhizosphere:soil ratios of these counts, however, indicate that at this point there is actually a greater selective action by roots in soil N than by those in soil X (29 and 15 respectively).

#### *Azotobacter*

As indicated earlier, numbers of *Azotobacter* were determined by spreading 1 ml. of a dilution uniformly over the surface of nitrogen-free mannite agar in petri plates. Actual counts may be obtained by drying the agar slightly after spreading the aliquot over the surface of the medium or by pouring the plates

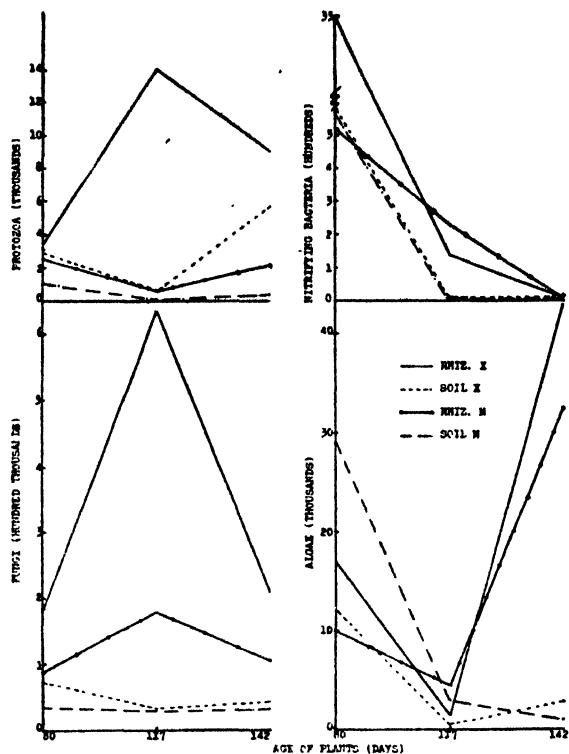


FIG. 2. EFFECT OF SOIL TREATMENT AND AGE OF PLANT ON NUMBERS OF SOIL ORGANISMS ON THE ROOT SURFACE OF MANGELS

TABLE 1

*Influence of age of plant on rhizosphere/soil ratios of numbers of different groups of soil organisms*

GROUP	SOIL N RHIZOSPHERE/CONTROL SOIL			SOIL X RHIZOSPHERE/CONTROL SOIL		
	80 days	117 days	142 days	80 days	117 days	142 days
Total bacteria.....	35	45	113	26	120	80
Actinomycetes.....	3	23	30	2	23	6
Nitrifying bacteria.....	0.9	2.2	..	6	1.4	2.0
Ammonifying bacteria.....	17	9	167	29	167	333
Denitrifying bacteria.....	43	15	2,000	29	230	2,000
Fungi.....	2.5	6	3	2.5	19	5
Algae.....	0.4	1.5	29	1.4	2.3	15
Protozoa.....	2.4	4	4.4	1.2	23	1.6
Azotobacter.....	0.4	5.3	1.9	0.9	1.5	2.0
Cellulose - decomposing bacteria.....	0.9	0.8	19.7	29.6	2.4	3.2
Anaerobic gas - producing bacteria.....	0.9	0.8	200	29	14	16
Anaerobic bacteria.....	19.4	39.7	28	11.5	46	7.6

4 days prior to making the test. This is a departure from the classical flask-dilution method or that involving spreading weighed amounts of soil on an agar or silica-gel medium (1, 20). For rhizosphere studies the dilution method is perhaps the only possible one unless root scrapings are used, a method which involves calculations on the basis of a mixture of soil and root tissue and which in consequence is not quite comparable to similar calculations based on non-rhizosphere soil. Perhaps even more important is the fact that the soil is much more highly dispersed by being shaken in water than when spread on agar plates

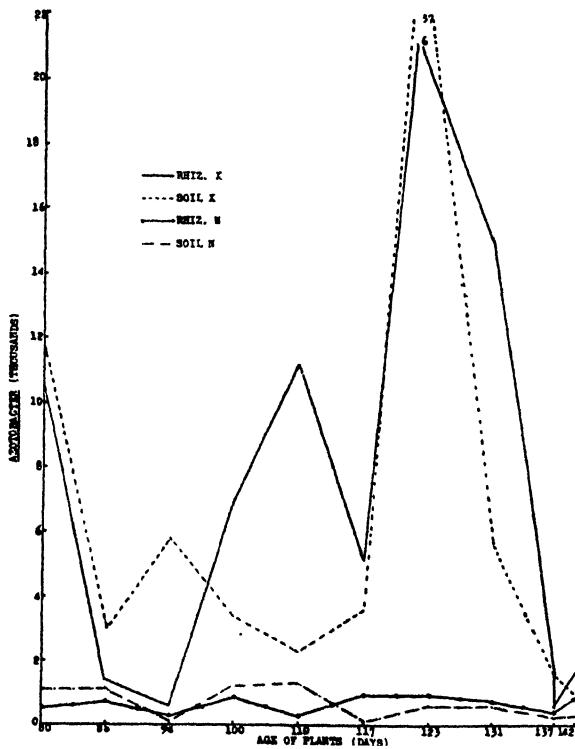


FIG. 3. INFLUENCE OF SOIL TREATMENT AND AGE OF PLANT ON NUMBERS OF *Azotobacter* IN THE RHIZOSPHERE OF MANGELS

directly. Counts are consequently higher and probably more accurate. In support of this, a sample of soil was diluted in the usual manner and 1-ml. aliquots were spread on the agar medium. At the same time, weighed amounts of soil were spread as uniformly as possible on agar medium in large (15-cm.) petri plates. Counts of 3,300 and 800 were obtained with the dilution-plate and the direct method respectively.

The dilution-plate method was selected instead of the dilution-flask method because of the ease of determining the presence of *Azotobacter* in the former. *Azotobacter* colonies have offered very little difficulty in identification, as was borne out by microscopic examination of large numbers. It is more difficult

and laborious to demonstrate the presence of *Azotobacter* in the "surface films" on a medium in flasks, and not infrequently confirmation is required by plating and isolation.

Numbers of *Azotobacter* were consistently greater in soil X and in the rhizosphere of plants growing in it than in the unfertilized soil N (fig. 3). This observation is somewhat at variance with the results obtained by Lochhead and

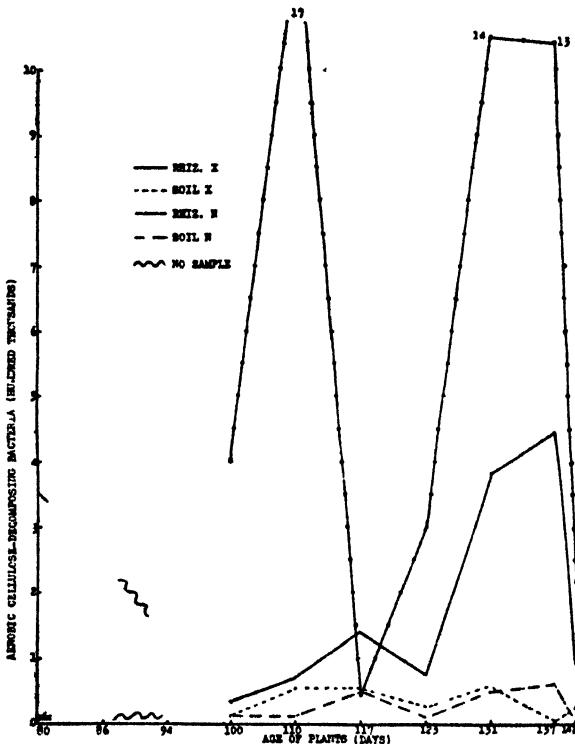


FIG. 4. "RHIZOSPHERE EFFECT" ON NUMBERS AS Affected BY SOIL TREATMENT AND AGE OF PLANT

Thexton (9) working with these same soils. There is no "rhizosphere effect," as such, although the organisms are certainly present on or near the root surface. Of possible significance is the slightly, but consistently, higher number of *Azotobacter* in the rhizosphere of plants in soil N after 110 days, coinciding perhaps with the increased vigor of these plants from this time on.

#### *Aerobic cellulose-decomposing bacteria*

Striking evidence of the influence of roots on numbers of aerobic cellulose-decomposing bacteria may be noted in figure 4. Even more interesting is the "rhizosphere effect" of plants in the unfertilized soil. Two maxima appear in rhizospheres of plants in both soils, but numbers are much greater on the roots in soil N.

TABLE 2  
Comparison of two media for growth of anaerobic bacteria producing gas

DILUTION OF SOIL	GAS PRODUCTION AFTER							
	3 days		5 days		7 days		13 days	
	Med. 11	Med. 12	Med. 11	Med. 12	Med. 11	Med. 12	Med. 11	Med. 12
1:100	0	+	+	+	+	+	+	+
1:500	0	+	+	+	+	+	+	+
1:1,000	0	+	+	+	+	+	+	+
1:5,000	0	+	+	+	+	+	+	+
1:10,000	0	+	0	+	+	+	+	+
1:50,000	0	+	0	+	+	+	+	+
1:100,000	0	0	0	0	0	0	0	+
1:1,000,000	0	0	0	0	0	0	0	0

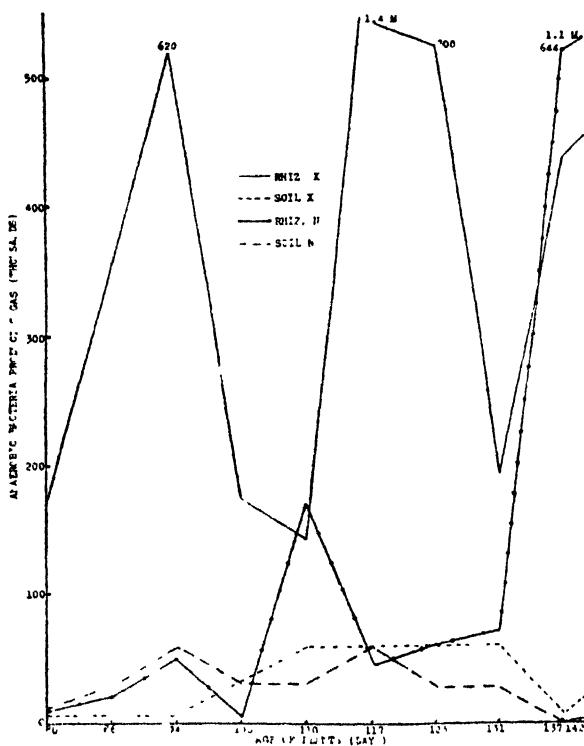


FIG. 5. EFFECT OF SOIL TREATMENT AND AGE OF PLANT ON COUNTS IN MANGEL RHIZOSPHERE

#### Anaerobic bacteria producing gas

These anaerobic bacteria are either very closely related to or are actually *Clostridium pasteurianum* types (2). In a comparison of Winogradsky's two media—glucose-peptone agar as used in this work and glucose-phosphate,

nitrogen-free agar (2, medium 11 and 12 respectively)—it was found (table 2) that gas production in both media occurred with approximately the same dilutions of soil after 7 days' incubation. It is likely therefore that the organisms developing in these media are nitrogen-fixing clostridia. Isolations support this view. The "rhizosphere effect" on this group of organisms is evident in the X series (fig. 5) throughout the growing period and may be explained on the

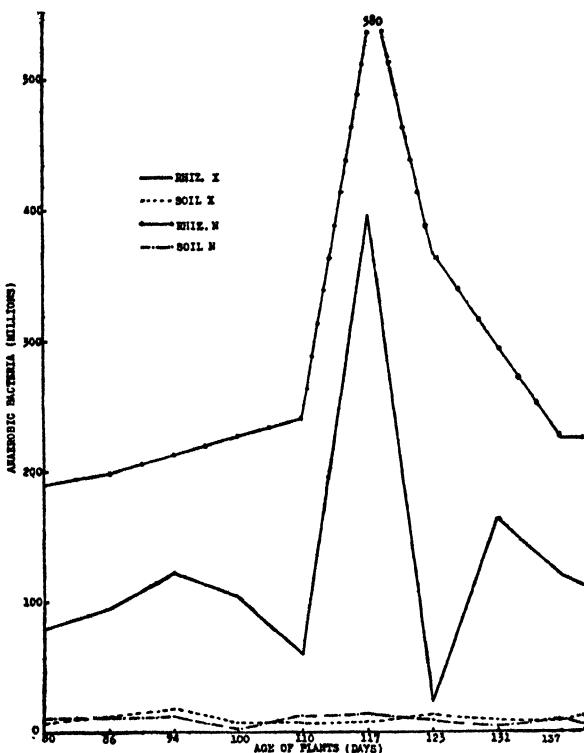


FIG. 6. PREVALENCE OF ANAEROBIC BACTERIA IN THE RHIZOSPHERE OF MANGELS

basis of extensive excretions of organic material which these bacteria can utilize. As the plants in soil N become larger and more vigorous functionally, they also begin to exert on these bacteria a selective action which becomes very marked at the end of the growing period. Rhizosphere:soil ratios at this period are much higher for these plants than for those in soil X (200 and 16 respectively).

#### *Anaerobic bacteria in general*

The tube method for determining numbers of anaerobic bacteria in general is admittedly not very satisfactory. The consistency of the results obtained (fig. 6) indicates, however, that this group of organisms requires further study. A distinct "rhizosphere effect" is evident, but the higher values in the N rhizospheres are at present inexplicable.

## DISCUSSION

The observed "rhizosphere effect" on numbers of bacteria is in accord with results obtained by most investigators. According to Starkey (14), Demidenko noted that organic substances were given off in large amounts "up to the time when assimilation processes reach a maximum." Starkey concluded that "microbial activity also follows a similar course of change, being greatest at periods of advanced vegetative development or fruiting." It is to be expected, then, that the response of this group of organisms to the vigorously growing and presumably actively excreting roots in the manured soil especially at 117 days would be particularly striking. The much slower increase in numbers of bacteria in the N-rhizosphere is explicable, therefore, on the basis of less vigorous plants which attain appreciable size and begin to manifest their full selective powers only after 117 days of growth. The effect of soil treatment is here apparently an indirect one in stimulating or retarding plant growth and thereby influencing the rhizosphere population.

Numbers of actinomyces in the rhizosphere are also affected by the age of plant and by soil treatment. Katznelson and Richardson (4) noted a rhizosphere:soil ratio of 8 in the rhizosphere of poorly growing greenhouse tomato plants in an infected soil, whereas a ratio of 66 was found on the root surfaces of healthy tomato plants in steam-sterilized soil after 3 months. Starkey (14) noted an increase in numbers on the root surfaces of plants in advanced stages of development. Sweet clover roots at 375 days had a negative "rhizosphere effect," but at 456 days a ratio of 8.6 was obtained. Young mangel beets and corn (at 66 and 113 days) had no selective action on this group and beans only a slight effect at 66 days.

Fungi were more numerous in the rhizosphere than in soil at a distance from roots, but as with actinomyces the "rhizosphere effect" was not so pronounced as for bacteria (table 1). The type of plant used, its age, soil treatment, soil reaction all affect this relationship. In his early work Starkey (12, 13) could not demonstrate a very significant increase of numbers of fungi on the roots of oats, corn, beans, and other plants, although he concluded that "numbers generally appear to be somewhat greater at the stages of extensive growth." In later work (14), however, he found consistently greater numbers of fungi on the surface of roots of table and mangel beets, sweet clover, beans, and corn than in soil 15 to 30 cm. away. Age of plant influenced rhizosphere:soil ratios appreciably; with sweet clover a ratio of 1.6 at 356 days was found to increase to 10 at 437 days. Katznelson and Richardson (4) obtained ratios as high as 36 on tomato roots in diseased greenhouse soil. Thom and Humfeld (17) obtained ratios of 5 and 11 with alfalfa and rye respectively; they also found that roots of corn growing in acid soil supported fungi in the ratio of 40 to 70 as compared with ratios of 2 to 5 in more alkaline soil. Timonin (18) working with flax seedlings, obtained ratios of 1.3 to 6.8, varying with plant variety and greater in drier than in moister soils. This was also the case with bacterial counts. It appears then that despite the admittedly unsatisfactory procedure of estimat-

ing numbers of fungi by the plate method, the results point to a definite "rhizosphere effect," which varies with the nature of the plant, its stage of maturity, nature of the soil, its treatment and moisture content.

Although a good deal of work has been done on the influence of specific crops on soil nitrates (15) there is no record, insofar as the writer is aware, of studies of numbers of nitrifying bacteria on root surfaces. The results obtained in this study are insufficient for discussion even though a ratio of 6 was obtained in the X-rhizosphere at 80 days. This is an appreciable effect. The intense activity of other organisms in the rhizosphere of vigorously growing plants may suppress these bacteria.

Ammonifying and denitrifying bacteria have not been studied extensively as groups. Krassilnikov *et al.* (7) found that the predominant forms in the rhizosphere consisted of nonsporing rods with pronounced ammonifying capacity. Krassilnikov (5) also reported that organisms such as *B. denitrificans* and *B. fluorescens* multiplied extensively on root surfaces. These results support the present observations of very large numbers of ammonifying and denitrifying bacteria in the rhizospheres of vigorously growing plants in soil X at 117 days and their increase on the roots of mature plants in both soils.

As with nitrifying bacteria, numbers of protozoa and algae in the rhizosphere have received virtually no attention. The stimulation exerted by roots in the manured soil on protozoa was very marked; a gradual increase in number of protozoa in the N-rhizospheres as the plants reached maturity also occurred. Of the protozoa, flagellates, ciliates, and amoebae were all represented, the last frequently in greatest number, especially in the rhizosphere. Algae attained a ratio of 29 and 15 in soil N and X respectively, numbers reaching 32,000 to 44,000. It may be of interest to note that the majority of these were green algae; blue-green forms appeared only in the first sample in the following numbers: soil N 280, rhizosphere N 2,580; soil X 60, rhizosphere X 350.

Although *Azotobacter* is present on mangel roots it does not appear to be preferentially stimulated, an observation supported by other investigators working with a variety of plants (8). According to the evidence presented in this paper, however, anaerobic nitrogen-fixing bacteria behave quite differently, finding the rhizosphere of vigorously functioning mangel roots (in the X soil throughout the growing period and in the N soil at 142 days) a very congenial habitat. According to Starkey (12) Velich and others found an organism, *Clostridium gelatinosum* Laxa, in greater abundance in soils close to the roots and on the root epidermis of beets, mangels, and other plants. The number of nitrogen-fixing clostridia observed in the present study, reaching as high as 1.4 million in the rhizosphere as compared with a maximum count of 36,000 for *Azotobacter*, suggests that the former might be a more important source of nitrogen for plants than the latter is considered to be by some (3, 8). Bezzsonoff and Truffaut [see Joffe, (3)] considered that *Clostridium pasteurianum* was involved in supplying nitrogen to corn. Lipman and Starkey (8) note, however, that Demidenko did not obtain a selective action on these bacteria by plant roots.

Krassilnikov *et al.* (6) reported striking increases in cellulose-decomposing

bacteria in the rhizosphere of certain plants. These same authors (7) noted a simultaneous increase of total numbers of microorganisms and aerobic cellulose-decomposing bacteria in the rhizosphere of sunflower and soybean as the plants developed; an increase in numbers in the early part of the growing period was followed by a drop and then by another peak, in the later part of the season. Calculations of rhizosphere:soil ratios from their results indicate a distinct "rhizosphere effect" rather than an environmental effect causing concomitant changes in numbers in both soil and rhizosphere. The above workers concluded that these organisms actively decompose dying-off parts of roots and that the products of this decomposition are subsequently used by other organisms. This explanation would not account for the greater numbers of these bacteria in the N-rhizosphere. Another possibility is that excretions of carbohydrates by the vigorous roots in soil X may inhibit these bacteria directly or may affect them indirectly by stimulating other forms whose metabolic products may inhibit the cellulose decomposers in the X-rhizospheres. Carbon dioxide produced by clostridia and other microorganisms may well suppress these aerobes.

It is clear that the rhizosphere is a unique environment exerting on many groups and species of soil organisms a powerful selective action which varies with the type, age, and vigor of a plant and the nature, treatment, and moisture content of the soil on which it grows. The soil factors may exert their influence directly on the organisms in the soil and rhizosphere or indirectly by stimulating or retarding plant development. The specific influences involved in this zone of interaction between roots and microorganisms have not been completely elucidated but may provide a basis for understanding various phenomena from improved plant growth to serious root injury. The fields of investigation which such problems open are of great interest both academically and practically.

#### SUMMARY

Mangel roots exerted a striking selective action on numbers of bacteria, actinomycetes, fungi, ammonifying and denitrifying bacteria, and protozoa in both manured and unfertilized soils. Roots in the former, supported greater numbers at the period of most vigorous growth, after which a decline occurred. More slowly growing plants (in the unfertilized soil) manifested their full selective power only when approaching maturity. Soil treatment apparently exerted its effect indirectly by influencing the rate of plant growth. A "rhizosphere effect" on algae was noted in the latter part of the growing season and on nitrifying bacteria during the earlier period.

By means of a dilution-plate technique it was demonstrated that *Azotobacter* were more numerous in the manured soil and on the roots growing in it than in unfertilized soil. No "rhizosphere effect" was apparent.

Aerobic cellulose-decomposing bacteria were preferentially stimulated by mangel roots and especially so by those growing in unfertilized soil.

Gas-producing clostridia (closely related to if not identical with *Clostridium pasteurianum*) were very abundant on roots in manured soil throughout the growing season and on roots in untreated soil as they reached maturity.

Anaerobic bacteria in general were consistently stimulated in the rhizosphere of plants in both fertilized and unfertilized soil and were always present in greater numbers on the roots in the latter.

#### REFERENCES

- (1) CURIE, I. H. 1931 A method for the study of *Azotobacter* and its application to fertility plot soils. *Soil Sci.* 32: 9-24.
- (2) FRED, E. B., AND WAKSMAN, S. A. 1928 Laboratory Manual of General Microbiology. McGraw-Hill Book Company, Inc., New York.
- (3) JOFFE, J. S. 1944 A pedologist views the nitrogen problem. *Soil Sci. Soc. Amer. Proc.* 8: 23-36.
- (4) KATZNELSON, H. K., AND RICHARDSON, L. T. 1943 The microflora of the rhizosphere of tomato plants in relation to soil sterilization. *Canad. Jour. Res. (C)* 21: 249-255.
- (5) KRASSILNIKOV, N. A. 1934 Influence of root excretion on the development of *Azotobacter* and of other soil microbes. *Microbiol. [U.S.S.R.]* 3: 343-359.
- (6) KRASSILNIKOV, N. A., KRISS, A. E., AND LITVINOV, M. A. 1936 The microbiological characteristics of the rhizosphere of cultural plants. *Microbiol. [U.S.S.R.]* 5: 87-98.
- (7) KRASSILNIKOV, N. A., KRISS, A. E., AND LITVINOV, M. A. 1936 The effect of the root system on the soil microflora. *Microbiol. [U.S.S.R.]* 5: 270-286.
- (8) LIPMAN, J. G., AND STARKEY, R. L. 1935 Broad relationships between microorganisms and soil fertility. *N. J. Agr. Exp. Sta. Bul.* 595.
- (9) LOCHHEAD, A. G., AND THEXTON, R. H. 1936 A four-year quantitative study of nitrogen-fixing bacteria in soils of different fertilizer treatment. *Canad. Jour. Res. (C)* 14: 166-177.
- (10) LOCHHEAD, A. G. 1940 Qualitative studies of soil microorganisms: III. Influence of plant growth on the character of the bacterial flora. *Canad. Jour. Res. (C)* 18: 42-53.
- (11) SMITH, N. R., AND DAWSON, V. T. 1944 The bacteriostatic action of rose bengal in media used for plate counts of soil fungi. *Soil Sci.* 58: 467-471.
- (12) STARKEY, R. L. 1929 Some influences of the development of higher plants upon the microorganisms in the soil: I. Historical and introductory. *Soil Sci.* 27: 319-334.
- (13) STARKEY, R. L. 1929 Some influences of the development of higher plants upon the microorganisms in the soil: II. Influence of the stage of plant growth upon abundance of organisms. *Soil Sci.* 27: 355-378.
- (14) STARKEY, R. L. 1931 Some influences of the development of higher plants upon the microorganisms in the soil: IV. Influence of proximity of roots on abundance and activity of microorganisms. *Soil Sci.* 32: 367-393.
- (15) STARKEY, R. L. 1931 Some influences of the development of higher plants upon the microorganisms in the soil: V. Effects of plants upon distribution of nitrates. *Soil Sci.* 32: 395-404.
- (16) TAYLOR, C. B., AND LOCHHEAD, A. G. 1938 Qualitative studies of soil microorganisms: II. A survey of the bacterial flora of soils differing in fertility. *Canad. Jour. Res. (C)* 16: 162-173.
- (17) THOM, C., AND HUMFELD, H. 1932 Notes on the associations of microorganisms and roots. *Soil Sci.* 34: 29-36.
- (18) TIMONIN, M. I. 1940 The interaction of higher plants and soil microorganisms: II. Study of the microbial population of the rhizosphere in relation to resistance of plants to soil-borne diseases. *Canad. Jour. Res. (B)* 18: 444-456.
- (19) WAKSMAN, S. A. 1932 Principles of Soil Microbiology, ed. 2. The Williams & Wilkins Co., Baltimore.
- (20) WINOGRADSKY, S. 1925 Sur une méthode pour apprécier le pouvoir fixateur de l'azote dans les terres. *Compt. Rend. Acad. Sci. [Paris]* 180: 711-716.

# EFFECT OF CROPPING ON THE NITROGEN, PHOSPHORUS, AND ORGANIC CARBON CONTENT OF A DRY-FARM SOIL AND ON THE YIELD OF WHEAT

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This investigation was started to determine the influence of cropping, rotations, and green manure practices on the nitrogen, phosphorus, and organic carbon content and the carbon-nitrogen ratio of the soil and on the yield of wheat on a dry-farm soil. The study is an outgrowth of earlier work (6) in which attempts were made to determine the influence of cropping upon the nitrogen content of the soil by sampling fields that had been cropped for varying periods and using adjacent virgin land for comparison. Reliability of the results in the earlier work depended on accuracy of the information obtained as to the cropping systems that had been followed and on reliability of the sampling and analyses. It was believed that by sampling soils near the beginning of the experiments and after 25 years of cropping under specific conditions on the same plots, some of the uncertainties encountered in the first work might partly be eliminated. This would leave reliability of sampling at the beginning and close of the experiment as the principal variable.

## PLAN OF THE EXPERIMENT

This investigation was conducted at the Nephi Dry-Land Substation 5 miles south of Nephi, Utah, on the north slope of Levan Ridge. The soil is a typical dry-farm clay loam derived from the weathering of the adjacent mountains, which contain deposits of phosphorus, potassium, and gypsum. When first cropped for experimental purposes, the surface foot contained approximately 3,600 pounds of total nitrogen, 8,000 pounds of total phosphorus, 8,600 pounds of total potassium, 1,480 pounds of total sulfur, 38,000 pounds of organic carbon, 69,000 pounds of magnesium carbonate, and 139,000 pounds of calcium carbonate. Thus the soil was well supplied with all these plant elements, with the possible exception of nitrogen, leaving nitrogen and moisture as the limiting factors of crop production. The average annual rainfall from 1903 to 1945 inclusive, was 12.6 inches.

## METHOD OF INVESTIGATION

There were eight treatments in the experiment, as outlined in table 1. Sagebrush (*Artemisia tridentata*) covered the experimental area previous to 1903, at which time the land was plowed. Crops were first grown in 1904. Plots under study were sampled in 1-foot sections in 1918 by making five borings at random on each plot to a depth of 3 feet. The five samples were mixed and a composite was used for analysis. Twenty-five years later samples were again

taken. This time, the foot sections of each of the five borings were analyzed separately and the results averaged. Total nitrogen was determined by the Kjeldahl method, and phosphorus and organic carbon were determined by the combustion (1) method. Results as reported are averages of pounds in one acre-foot of soil.

TABLE 1

*Nitrogen content of soil of variously treated plots at beginning of sampling in 1918 and after 25 years*

Weight of 1 acre-foot of soil considered as 3,600,000 pounds

TREATMENT	TIME	NITROGEN, POUNDS PER ACRE							Change in content per cent
		1st Foot	2nd Foot	3rd Foot	Total	Loss or gain	Amount removed by crop	Annual loss or gain	
Continuous wheat, 1904-1943	Beginning	3600	3204	2196	9000	-396	214	-15.8	-4.40
	After 25 years	3240	3060	2304	8604				
Alternate cropping, 1904-1942	Beginning	3420	2952	2448	8820	-684	218	-27.4	-7.75
	After 25 years	3240	2592	2304	8136				
Two crops, one fallow 1904-1943	Beginning	3420	2952	2448	8820	-756	211	-30.2	-8.57
	After 25 years	3240	2736	2088	8064				
One crop, two fallow, 1904-1943	Beginning	3440	2736	2808	8984	-1784	183	-71.4	-19.85
	After 25 years	2808	2412	1980	7200				
Potatoes alternating with wheat, 1915-1943	Beginning	3528	3456	2376	9360	-108		-4.3	-1.15
	After 25 years	3600	3276	2376	9252				
Peas alternating with wheat, 1913-1943	Beginning	3240	2716	2376	8352	+72		+2.9	+0.86
	After 25 years	3420	2808	2196	8424				
Peas plowed under as green manure, 1916-1943	Beginning	3204	2520	2448	8172	-36	240	-1.4	-0.44
	After 25 years	3240	2556	2412	8208				
Permanent fallow, 1916-1943	Beginning	3420	3168	3240	9828	-1404		-56.2	-14.3
	After 25 years	3240	2628	2556	8424				

The pounds of nitrogen in the first, second, and third foot at the beginning and end of the experiment are given in table 1. These data show considerable variation in the nitrogen content of the soil on the various plots at the beginning and likewise in 1943 when the last samples were taken. The nitrogen changes varied from an annual loss of 71.4 pounds for one crop in three years, to an annual gain of 2.9 pounds where peas were alternated with wheat. Permanent fallow showed the next highest loss, amounting to 56.2 pounds per acre annually.

It is evident from these results, together with those obtained in a study of irrigated soil (3), that fallowing is more wasteful of soil nitrogen than is continuous cropping. Peas alternating with wheat apparently supplied the nitrogen need of the crop, thus tending to maintain the nitrogen content of the soil. Plots on which peas were plowed under as a green manure crop showed a total loss of only 36 pounds per acre, but the wheat crop had utilized 240 pounds per acre showing that this treatment also tended to maintain the nitrogen balance. Total nitrogen lost from plots grown to wheat only was much more than that removed by the growing crops. This substantiates earlier findings (2). The soil cropped continuously to wheat lost 15.8 pounds nitrogen per acre annually, whereas soil continuously in oats and irrigated lost over 300 pounds per acre (3). Continuously fallow irrigated soil lost 252 pounds nitrogen per acre. Irrigated soil on which alfalfa was grown continuously lost 106 pounds nitrogen per acre (3). This raises the question of whether or not dry-farm soil cropped continuously to alfalfa would register gains in soil nitrogen. Irrigated soil richer in nitrogen did not. Unpublished data indicate that dry-farm soil in close proximity to the Nephi Dry-Land Station on which alfalfa was grown as a first crop following removal of the brush did maintain the nitrogen content without loss over a 10-year period when adjacent virgin soil was used for comparison.

In an earlier study (2) of the nitrogen content of cropped land as compared with adjacent virgin land in 20 fields in Juab Valley the annual loss varied from 13 pounds per acre on individual fields up to 97 pounds and with an average annual loss of 45 pounds per acre. The average annual loss in the present study of the four cropping tests amounted to 28.7 pounds per acre.

Total phosphorus of the soil on the plots studied varied considerably, as indicated in table 2. Annual loss, however, was not excessive from any of the treatments. Loss of phosphorus from irrigated land studied earlier was only slightly greater than that from the soil included in these tests. This lends support to the conclusion that the phosphorus lost as a result of leaching from either this dry-farm soil or the irrigated soil is insignificant. It is interesting to note that at such a rate of loss it would require approximately 200 years to remove the equivalent of the quantity of phosphorus occurring in the surface acre-foot; but the roots of wheat grown on dry-farm soil may penetrate the soil to a depth of 6 or more feet.

The total amounts of organic matter ( $C \times 1.724$ ) found in the soil of the variously treated plots are given in table 3. According to these data, permanently fallowed soil lost the greatest amount of organic matter, 318 pounds per acre annually. Plots which produced two crops in 3 years lost the next greatest amount, 119 pounds yearly. All other treatments resulted in gains, ranging from 11 pounds annually for one wheat crop and two fallows up to 272 pounds yearly for wheat alternating with potatoes.

In the light of the results with nitrogen, it is not surprising that organic matter increased on plots grown to peas alternating with wheat, but increases for plots cropped continuously, alternately, and once in 2 years are quite different from

the annual yearly loss of 576 pounds of organic matter from cropped land in the same general area using virgin areas for comparison (2), and are also contrary to results obtained by Sievers and Holtz (5) and Russel (4). They are in keeping, however, with earlier data by Stewart (6). A binder was used in harvesting the winter wheat, leaving stubble varying from 6 to 10 inches in

TABLE 2

*Phosphorus content of variously treated plots at beginning of sampling in 1918 and after 25 years*

Weight of 1 acre-foot of soil considered as 3,800,000 pounds

TREATMENT	TIME	PHOSPHORUS, POUNDS PER ACRE					
		1st Foot	2nd Foot	3rd Foot	Total	Loss or gain	Annual loss or gain
Continuous wheat, 1904-1943	Beginning	9684	9612	9712	29008		
	After 25 years	9108	10044	9468	28620	-388	-16
Alternate cropping, 1904-1942	Beginning	8604	9576	8076	26256		
	After 25 years	8352	9252	8100	25704	-552	-22
Two crops, one fallow, 1904-1943	Beginning	7884	8208	8216	25308		
	After 25 years	7668	8568	7884	24120	-1188	-47
One crop, two fallow, 1904-1943	Beginning	8704	8308	9100	26112		
	After 25 years	8684	8280	9008	25972	-140	-6
Potatoes alternating with wheat, 1915-1943	Beginning	8352	9216	10764	28332		
	After 25 years	8028	8856	10440	27324	-1008	-40
Peas alternating with wheat, 1913-1943	Beginning	8712	8856	9672	27240		
	After 25 years	8316	8712	9612	26640	-600	-24
Peas plowed under as green manure, 1916-1943	Beginning	7452	7164	6724	21340		
	After 25 years	7380	7452	6300	21132	-208	-47
Permanent fallow, 1916-1943	Beginning	7524	6868	8064	22456		
	After 25 years	7344	7632	6804	21780	-676	-27

height. With this comparatively small amount of straw returned to the soil it is not clear why the soil of these plots failed to show losses.

Since most of the treatments in this study resulted in increase in organic matter over the 25-year period, it follows that the carbon-nitrogen ratio would become wider. The results are given in table 4. In a previous study (4) the carbon-nitrogen ratio of virgin soil adjacent to 20 farms in Juab Valley was 10.1:1 and that for cropped soils was 9.55:1, indicating a narrowing of the ratio with cropping. Similar results have been obtained by other workers (5).

The primary purpose of the tests as outlined involving chemical analyses was to determine the effect of the various treatments on yield of winter wheat.

As indicated by table 5 the cropping tests were started in 1903 when the land was first broken out of sagebrush and the first crop was harvested in 1904. Winter wheat alternating with field peas and with potatoes began in 1913. Peas plowed under for green manure alternating with winter wheat and permanent fallow were started in 1916. All of these later tests were established on land

TABLE 3

*Organic matter content ( $C \times 1.724$ ) of variously treated plots at beginning of sampling in 1918 and after 25 years*

Weight of 1 acre-foot considered as 3,600,000 pounds

TREATMENT	TIME	ORGANIC MATTER, POUNDS PER ACRE					
		1st Foot	2nd Foot	3rd Foot	Total	Loss or gain	Annual loss or gain
Continuous winter wheat	Beginning	56948	52568	40217	149733		
	After 25 years	61443	53809	39287	154539	4806	192
Alternate cropping to winter wheat since 1904	Beginning	58388	45824	39907	144116		
	After 25 years	65167	44314	41148	150699	6583	261
Two crops, one fallow, since 1904	Beginning	59547	46500	34845	140892		
	After 25 years	59395	44996	33515	137906	-2986	-119
One crop, two fallow, since 1904	Beginning	64629	55982	45825	166436		
	After 25 years	64795	56726	45183	166704	268	11
Potatoes alternating with winter wheat	Beginning	58899	51823	44748	150470		
	After 25 years	64174	53809	43507	161490	11020	272
Peas alternating with winter wheat	Beginning	57409	50148	41459	149016		
	After 25 years	61940	49092	38356	149388	372	15
Peas plowed under as green manure, alternating with winter wheat	Beginning	63057	39224	32956	135237		
	After 25 years	59581	40776	40279	140634	5397	216
Permanent fallow	Beginning	54057	52320	43321	149698		
	After 25 yrs.	52134	53003	36618	141755	-7943	-318

which was first plowed in 1903. Means within these three experiments are directly comparable.

In the cropping test (table 5), the highest mean acre yield of 24.1 bushels over the 41 years was produced from one crop in 3 years, alternate crop and fallow with an average of 23.4 bushels per acre was second, two crops in 3 years producing an average yield of 16.8 bushels was third, and continuous cropping with an average yield of 9.98 bushels an acre was last. Considering the total yields of a single replication from 1904 to 1945, inclusive, one crop in 3 years produced

a total of 337.2 bushels to an acre, alternate cropping 507.3 bushels, two crops in 3 years 485.2 bushels, and continuous cropping 435.9 bushels. In the early period of dry farming, all of these cropping methods were tried in Utah, but alternate crop and fallow was found to be most successful and profitable in areas of limited rainfall.

Check plots in the rotation and green manure tests were not included in the study of chemical changes of the soil, but mean yields from these plots were added for the purpose of making comparisons.

TABLE 4

*Carbon-nitrogen ratio in soil of variously treated plots at beginning of sampling in 1918 and after 25 years*

TREATMENT	TIME	1ST FOOT	2ND FOOT	3RD FOOT
Continuous wheat	Beginning	9.2:1	9.5:1	9.9:1
	After 25 years	11.0:1	10.2:1	9.5:1
Alternate cropping since 1904	Beginning	9.9:1	9.0:1	11.7:1
	After 25 years	11.7:1	9.9:1	10.4:1
Two crops, one fallow, since 1904	Beginning	10.1:1	9.1:1	8.5:1
	After 25 years	10.6:1	9.5:1	9.3:1
One crop, two fallow, since 1904	Beginning	9.0:1	11.6:1	9.5:1
	After 25 years	13.4:1	13.6:1	13.2:1
Potatoes alternating with wheat	Beginning	9.8:1	9.7:1	10.9:1
	After 25 years	10.3:1	9.5:1	10.6:1
Peas alternating with wheat	Beginning	10.3:1	10.6:1	10.1:1
	After 25 years	10.5:1	10.1:1	10.1:1
Peas plowed under as green manure	Beginning	11.4:1	9.0:1	7.8:1
	After 25 years	10.7:1	9.3:1	9.7:1
Permanent fallow	Beginning	9.7:1	9.6:1	7.8:1
	After 25 years	9.3:1	11.7:1	8.3:1

In the rotation test (table 5) the yield of winter wheat following fallow produced in excess of 3 bushels to an acre more than winter wheat rotated with peas or potatoes. This difference was found to be statistically significant.

In the comparison of winter wheat grown after fallow and winter wheat following peas plowed under as a green manure crop, the wheat-fallow combination outyielded the wheat-pea by 1.77 bushels per acre. When all of the data in this test involving a number of other treatments were analyzed, this difference was found to be significant.

Regression coefficients were calculated for all but two of the tests. The regression coefficients of yields for wheat following peas as well as potatoes were positive in each case; in other words, yields tended to increase with time. With

wheat following fallow, the regression coefficient was negative. None, however, differed significantly from zero.

In the green manure test, the regression coefficient for yields of wheat after fallow and that for wheat following peas were positive, but again neither one was statistically significant. The positive regression coefficient for wheat-fallow cropping can be accounted for by the fact that yields were low for a succession

TABLE 5

*Regression coefficients, mean acre yields, and correlations calculated for part or all of such tests as cropping systems, rotations, and green manure treatment at the Nephi Dry-Land Station, Nephi, Utah*

CROPPING SYSTEMS	REGRESSION COEFFICIENT <i>bushels</i>	BEGINNING OF REGRESSION CURVE <i>bushels</i>	END OF REGRESSION CURVE <i>bushels</i>	MEAN YIELD <i>bushels</i>	CORRELATION YIELD-RAINFALL
<i>Cropping treatment</i>					
Alternate crop and fallow, 1904-1945	-0.05	24.4	22.3	23.40	0.339*
Continuous cropping, 1904-1945	-0.146**	13.0	6.9	9.98	0.360*
One crop wheat in 3 years, 1904-1945				24.10	
Two crops wheat in 3 years, 1904-1945				16.80	
<i>Rotation</i>					
Wheat after fallow (check), 1913-1945	-0.113	24.93	21.32	23.13	
Wheat after peas, 1913-1945	0.045	19.29	20.73	20.00	
Wheat after potatoes, 1913-1945	0.030	19.42	20.38	19.90	
<i>Green manure treatment</i>					
Wheat after fallow (check), 1916-1945	0.107	20.95	24.05	22.47	
Wheat after peas for green manure, 1916-1945	0.072	19.64	21.80	20.70	
Rainfall, 1904-1944	-0.035	13.30	11.87	12.60	

\* Significant.

\*\* Highly significant.

of seasons beginning with 1916, thus starting the regression curve out at a lower level followed by an upward trend as rainfall became more favorable.

The regression coefficients for alternate and continuous cropping and rainfall are also given in table 5, and the respective regression curves are shown in figure 1. As indicated in figure 1, alternate cropping varied in yield from 7.2 bushels per acre in 1913 up to 45.9 bushels in 1926. Continuous cropping, producing at a lower level, ranged from a low of 2 bushels in 1919 to a high of 24 bushels in 1914. The annual rainfall varied from 7 inches in 1931 to 21 inches in 1941. Slopes of these three regression curves point downward from the horizontal, but the only regression coefficient (table 5) differing significantly from zero is

that for continuous cropping. The regression curve for alternate cropping began in 1904 at 24.4 bushels to an acre and ended in 1945 at 22.3 bushels. Continuous cropping started at 13 bushels and ended at 6.9 bushels. Precipitation for the same 41-year period began at 13.3 inches per year and ended at 11.87.

As indicated by the data in tables 1, 2, and 3, plots cropped continuously lost less nitrogen, phosphorus, and organic matter than plots cropped alternately,

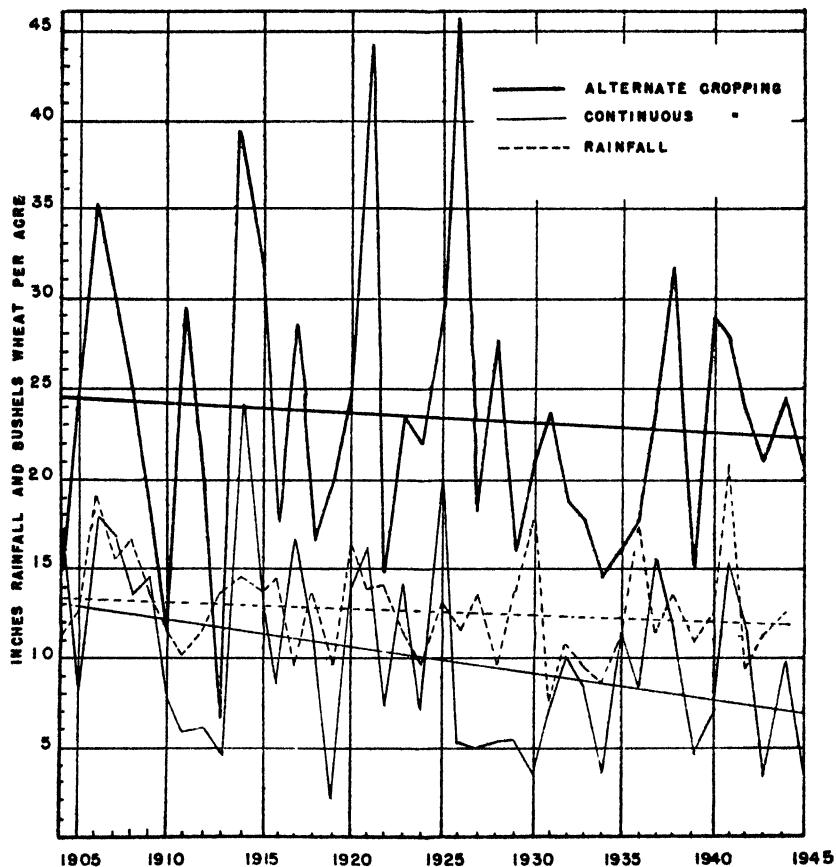


FIG. 1. YIELDS OF WHEAT IN BUSHELS PER ACRE FROM CONTINUOUS AND ALTERNATE CROPPING AND INCHES OF RAINFALL TOGETHER WITH THE RESPECTIVE REGRESSION LINES

Data collected at the Nephi Dry-Land Station, 1904 to 1945 inclusive

yet the production trend of the former differed significantly from zero. The reason for this is not well understood.

Correlations between rainfall and winter wheat yields obtained from alternate and continuous cropping were calculated (table 5). The correlation between alternate cropping and rainfall was 0.339 and for continuous cropping 0.360. Both were statistically significant. The correlation between yields of alternately and continuously cropped plots amounted to 0.46 and was highly sig-

nificant. Although the correlations between total annual rainfall and yield were both statistically significant, yet both were too low to be of much value for predicting crop yields.

Without question, moisture is the most important factor controlling production, but distribution of rainfall is more important than total amount. From the data in figure 1 it is obvious that in such seasons as 1906, 1907, 1914, 1920, 1921, 1925, and 1941 rainfall and wheat yields were all above normal, tending to vary in the same direction. In 1913, the annual rainfall was above the mean, but alternate cropping produced only 7.2 bushels to an acre, and continuous cropping 4.5 bushels. The other extreme occurred in 1926 when the wheat yield on alternately cropped plots reached 45.9 bushels to an acre and the rainfall totaled 11.67 inches for the year. Wheat yield was 196 per cent of the mean, and rainfall was only slightly over 93 per cent of normal.

High yields under dry-farm conditions depend upon a fallow high in available plant nutrients plus emergence of the plants early enough in fall to allow tillering before winter. Under such conditions, plants are usually in full head by June 1, or soon thereafter. Winter or early spring emergence is usually followed by late heading. This forces the plant to make a part of its vegetative growth during the heat of early summer, thus markedly reducing chances for average or above average yields. High winter or high spring rainfall ordinarily is not conducive to high wheat yields when the stand is suffering from a poor start.

#### SUMMARY

A group of selected plots included in a cropping test, rotation experiment, and a green manure test treated in the same manner since 1904, 1912, and 1916, respectively, were first sampled in 1918 and 25 years later in 1943 for the purpose of determining changes in total nitrogen, phosphorus, and organic matter. Winter wheat yields from the beginning of the respective tests are also reported. A summary of the results of this study follows:

All treatments resulted in losses of nitrogen except where peas were rotated with winter wheat. Potatoes alternating with wheat in rotation showed only a slight loss. Both pea treatments appeared to maintain the nitrogen of the soil at or near equilibrium. The greatest loss of nitrogen occurred on plots cropped every third year with two clean intervening fallow periods. This was followed in order by permanent fallow, two crops in 3 years, alternate wheat and fallow, with continuous wheat lowest of the cropping tests.

In the cropping test, loss of nitrogen from the soil was much greater than the amount utilized by the crop.

All plots lost phosphorus beginning with two crops and a fallow, which was highest (1188 pounds to an acre), and ending with peas plowed under as green manure, which was lowest (208 pounds). Other treatments varied between these two extremes. As compared to total amount of phosphorus in the soil, these losses are considered insignificant.

Organic matter losses occurred only on plots in permanent fallow and on plots cropped 2 years with one intervening fallow; all others showed gains. This is contrary to results obtained in other investigations. Since loss of nitrogen occurred on all wheat plots and most of the same plots showed gains in organic matter, obviously, according to the data, this resulted in a wider carbon-nitrogen ratio.

In a comparison of total production of winter wheat in the cropping test over the 41-year

period, alternate wheat and fallow produced the greatest total yield, followed by two crops in 3 years, then continuous cropping, with one crop in 3 years last.

In the rotations with winter wheat alternating with fallow, peas, and potatoes, the standard wheat-fallow combination produced significantly higher yields than wheat following either one of the intertilled crops.

Wheat yields were significantly higher after fallow than when plowed under as a green manure crop.

Regression coefficients calculated for alternate and continuous cropping of winter wheat were both negative, only the latter differing significantly from zero.

In the rotation as well as in the green manure tests, none of the regression coefficients indicated significant changes in yields over the period of the test.

The regression curve for yearly rainfall covering the period from 1904 to 1945, inclusive, sloped downward from the horizontal, but the deviation was not significant.

Correlations between wheat yields produced on alternately cropped and continuously cropped plots and total yearly rainfall were statistically significant but small. This seems to indicate that seasonal distribution of precipitation is more important than total amount.

#### REFERENCES

- (1) Association of Official Agricultural Chemists. 1940 Official and Tentative Methods of Analysis. Washington, D. C.
- (2) BRACKEN, A. F., AND GREAVES, J. E. 1941 Losses of nitrogen and organic matter from dry-farm soils. *Soil Sci.* 51: 1-15.
- (3) GREAVES, J. E., AND HIRST, C. T. 1943 The influence of cropping on the nitrogen, phosphorus and organic matter of the soil under irrigation farming. Utah Agr. Exp. Sta. Bul. 310.
- (4) RUSSEL, J. C. 1929 Organic matter problems under dry-farming conditions. *Jour. Amer. Soc. Agron.* 21: 960-969.
- (5) SIEVERS, F. J., AND HOLTZ, H. F. 1922 The silt loams of eastern Washington and their management. Wash. Agr. Exp. Sta. Bul. 166.
- (6) STEWART, R. 1910 The nitrogen and humus problems in dry-land farming. Utah Agr. Exp. Sta. Bul. 109.

# A LABORATORY STUDY OF LYSIMETER DRAINAGE UNDER CONTROLLED SOIL MOISTURE TENSION<sup>1</sup>

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The provision of adequate soil drainage presents one of the important problems which must be solved by students of soil and vegetation in studies involving the use of lysimeters. Until relatively recently the abnormal soil moisture condition existing in lysimeters provided with the usual soil-over-air seepage interface was not recognized as a factor having a significant bearing upon the quality of results obtained from these instruments. Within the last 10 years, however, several critical studies of this subject have been made, and the matter has received recognition and a considerable amount of discussion in the literature. Aderkas (1) found that when shallow lysimeters were drained they retained more water per unit depth of soil than did those of greater depth. This he attributed to the soil/air "interface" located at the drainage plane. Neal, Richards, and Russell (6) buried tensiometers at several depths in a confined lysimeter and, although no drainage occurred during the course of their study, they concluded that the soil at the bottom of the lysimeter would of necessity be very nearly saturated before drainage could start. In 1940 and 1941 the reports of the committee on evaporation and transpiration of the American Geophysical Union (3) were devoted almost exclusively to discussions of lysimeters. Considerable emphasis was placed upon the need for normal soil moisture conditions within enclosed soil blocks if the results obtained from them were to be applicable under natural soil conditions in the field.

The lysimeter installation of the San Dimas Experimental Forest<sup>2</sup> provided an opportunity to compare soil water and plant growth conditions over a 3-year period in four kinds of lysimeters varying in soil depth and drainage facilities, although identical in soil type and species of vegetation. This study showed a marked influence of soil depth, in the range from 24 to 72 inches, upon the annual soil water cycle and upon the dry weight of plant material produced. It showed further that no consistent relationship exists between the annual soil water cycle of lysimeters having soil/air drainage interfaces and those in which the lysimeter soil water is in capillary contact with the soil beneath.<sup>3</sup>

These reports indicate the need for modification of the generally accepted method of lysimeter drainage for the purpose of removing the excess moisture

<sup>1</sup> The laboratory phase of this study was carried on by W. B. Hanawalt and C. R. Burek of Civilian Public Service camp #76, Glendora, California, whose aid is gratefully acknowledged.

<sup>2</sup> Colman, E. A., and Hamilton, E. L. The San Dimas lysimeters. [Unpublished.] Calif. Forest and Range Experiment Station, 1944.

<sup>3</sup> Colman, E. A., and Hamilton, E. L. A study of the relative performance of four types of lysimeters. [Unpublished.] Calif. Forest and Range Experiment Station, 1944.

in the soil layer lying above the soil/air interface. In a simple yet highly suggestive experiment Wallihan in 1940 demonstrated the possibility of draining lysimeter soil through a porous fired-clay cup held under constant tension (8). His lysimeters were relatively small (12-inch diameter cylinders, 30 inches deep), but his results were striking nonetheless. By maintaining a tension of 10 cm. of mercury on the water in the clay cup he brought about a reduced rate of evaporation from the soil surface, a reduced growth of plants, and an increased volume of drainage. His conclusion was that "since this (tension drainage) procedure rendered moisture conditions more nearly like those in naturally well drained soils . . . the abnormal moisture conditions in the ordinary type of lysimeter cause important errors in lysimeter studies," and he suggested drainage under tension as a means of providing more nearly natural soil conditions.

Tensiometers installed at the seepage planes of both the large and the unconfined San Dimas lysimeters, coupled with a 3-year record of soil moisture conditions within the soil blocks, have shown soil water differences there which can be attributed to the seepage facilities provided in these two installations. Seepage water must leave the large confined lysimeters by passing across a soil/air interface at the 6-foot depth, while the soil is continuous above and below this depth in the unconfined lysimeters. Seepage has never been observed in the large lysimeters when the pressure potential at the seepage plane has been less than zero. At the same depth in the unconfined lysimeters, however, pressure potentials as low as -50 gm. cm./gm.<sup>4</sup> have been observed regularly during the period when downward water movement across this plane was known to be taking place. Periodic soil moisture sampling in both kinds of lysimeter has shown that at the end of the seepage period the soil in the large lysimeters is saturated to a considerable height (at times as high as 3 feet) above the seepage plane, while no moistures greater than field capacity are found at the end of seepage in the unconfined lysimeter soil.

These observations led to a study of methods of eliminating the abnormal moisture condition in confined lysimeters. The method which appeared most promising involved the use of moisture tension apparatus by which the pressure potential at the seepage plane could be maintained, during the seepage period, at a value which would ensure the continuation of seepage until the soil at that plane would have reached its field capacity. Wallihan (8) had already demonstrated the possibility of reducing moistures by this method in small soil blocks, but the practicability of its application to long soil columns had not been shown, nor had the choice of proper tension values been made. It is the purpose of this paper to present the results of a laboratory study in which long soil columns were drained under several moisture tensions and the drained soil moisture condition was related to the saturation and field capacity values of the soil.

<sup>4</sup> A pressure potential of -1 gm.cm./gm. is equivalent to a moisture tension of 1 cm. of water. Pressure potential is expressed in units of energy while soil moisture tension is its negative pressure equivalent, and is expressed in terms of the height of a water column of equivalent weight per unit cross-sectional area.

## METHOD OF STUDY

The soil employed in this study was part of that used to fill all lysimeters of the San Dimas installation. It is a clay loam derived from metamorphosed



FIG. 1. TUBE, TENSIOMETERS, AND DRAINAGE TENSION CONTROL EQUIPMENT USED IN STUDYING LYSIMETER DRAINAGE

diorite and probably is most closely related to the Holland and Sierra soil series. As placed in the lysimeters it consists of 60 per cent sand, 25 per cent silt, and 15 per cent clay of which all but 3 per cent is colloidal. Both its moisture equivalent and field capacity are 19 per cent, and its wilting point is 7 per cent.

The soil was packed, air-dry, in a brass tube 6 inches in inside diameter and 7 feet long (fig. 1). Rings of  $\frac{3}{4}$ -inch holes had been drilled through the tube at

6-inch intervals from one end to the other to provide access to the soil for the placement of tensiometers and for obtaining moisture samples. These holes were covered with friction tape when not in use. The tube was held in the vertical position and the soil poured in slowly while a long sharpened steel rod was struck repeatedly as far as possible into the soil in the tube. The agitation and tamping thus accomplished served to settle the soil and minimize textural and structural layering. The tube was filled to a height of 74 inches. The apparent density of 1.56 calculated for the soil column falls midway between the average values of 1.57 and 1.55 determined for the large and the unconfined lysimeters respectively.

In the studies to be reported the tube was filled twice. Following the first filling (soil column A) the soil was irrigated and drained under moisture tensions of 0 and 50 cm. of water ( $-50$  gm. cm./gm. pressure potential). After being emptied and refilled (soil column B) and again irrigated, it was drained under tensions of 55 and 160 cm. of water. Although the average apparent soil densities were sensibly equal in both columns, the rates of seepage and the pressure potentials observed in the columns while under irrigation indicated a lower average permeability in column B than in column A. As will be brought out in the analysis of the pressure potential:moisture relationships, the lower part of column B appears to have been less permeable than the rest of the column, thus influencing the rate of water flow through this soil. In some respects, therefore, the two soil columns cannot be considered to present identical soil conditions, yet the influence of the several drainage tensions upon the draining and drained soils is nonetheless definite.

When the soil was drained under zero tension, thus simulating ordinary lysimeter conditions, the soil rested on a perforated sheet metal plate and seepage water was caught and measured by a 1 cubic inch tipping bucket of the type used in rate-recording rain-gages. When the soil was drained under tension, a porous fired clay plate 4 inches in diameter sealed to the rim of a glass funnel was pressed against the base of the soil column. The same porous plate was used in columns A and B. The funnel was filled with water and moisture tension was maintained either from a partly evacuated carboy or by the weight of a water column of suitable length hanging from the funnel. In the former case seepage was collected under tension in a sealed graduated cylinder. In the latter seepage dripped into the tipping bucket mentioned above, the seepage rate being recorded on a strip chart recorder.

A typical drainage trial was made as follows: The soil column, prepared as described above, was set vertically in a basement stair-well in which the temperature varied only 3 or 4°C. The soil was irrigated under a constant small hydrostatic pressure, and as the infiltrating water passed each ring of holes in the tube a tensiometer was installed at that depth. Each tensiometer consisted of a porous fired-clay tube 4 inches long and  $\frac{1}{2}$  inch in diameter connected to a differential mercury manometer so that both positive and negative pressure potentials could be measured. A moisture sample was taken from each ring of holes as soon as possible after the start of seepage. Irrigation was terminated

when relatively constant seepage rates had been attained, at which time a second set of moisture samples were taken and the top of the tube covered with a pliofilm sheet in order to minimize evaporation during the subsequent drainage period. The soil column was irrigated for more than 2 weeks in each phase of the study. During the irrigation and drainage period, daily or more frequent measurements were made of seepage and of pressure potentials at all levels. At the end of drainage, indicated by the lack of a measurable seepage increment over a period of several days, a final measurement of the pressure potential distribution was made and the final soil moisture distribution determined.

The drainage tensions of 0, 50, 55, and 160 cm. of water were selected on the basis of observed lysimeter soil characteristics. Zero tension corresponds to the minimum pressure potential observed during seepage at the bottom of the large confined lysimeters. It represents, in fact, the theoretical pressure potential at the soil/air seepage interface of an ordinary lysimeter when seepage has just ceased.

The 50-cm. tension was selected as equivalent to a pressure potential of -50 gm. cm./gm., which was the maximum observed in tensiometers at a depth of 6 feet in the unconfined lysimeters when downward water movement had virtually ceased. The moisture content at that depth was then close to 19 per cent already noted as representing the field capacity of this soil. Since the soil column in and below this lysimeter is continuous, the drained moisture distribution in the unconfined lysimeter is considered to be a fair approximation of that in a well-drained natural soil of the same kind. The 50- and 55-cm. drainage tensions were thus selected as being in the range of tensions observed at the 6-foot depth in an unconfined lysimeter when the soil at that depth was approximately at its field capacity.

The 160-cm. drainage tension was selected in the following manner: Laboratory determinations had been made of the relationship between pressure potential and moisture by draining 100 gm. blocks of lysimeter soil to constant moisture on a porous fired clay plate maintained at a constant moisture tension. It was observed that a tension of approximately 160 cm. of water was required to drain this soil to 19 per cent moisture. The difference of 110-cm. tension between the field and laboratory determinations has not yet been explained but it may possibly be related to the existence of a structure in the unconfined lysimeter soil which was not present in the laboratory-packed samples. The 160-cm. tension was employed in draining one of the soil columns, since these columns represented laboratory-packed soils more closely than they did 7-year-old lysimeter soils.

## RESULTS

### *Influence of drainage tension on seepage rates during irrigation period*

In figure 2 is shown seepage rate (inches depth of water per hour) in relation to time during both the irrigation and the drainage periods of the study. Seepage rates early in the irrigation period are not shown, but the plotted data cover a period long enough to show the rates characteristic of each trial. It

will be observed that there is a tendency for seepage rates to decrease with time but that the decrease over the period plotted is not great. This change in rate with time has been observed by Bodman (2) and others and has been attributed to leaching of the soil, dispersion and reorientation of colloid particles, and changes in soil pore configuration.

Two levels of seepage rates during the irrigation period are apparent in figure 2. The lower of these (about 0.02 inch per hour) is shown when the soils were drained under 0 and 55 cm. tension. The higher rate (0.05 inch per hour) is attained under 160 and 50 cm. tension. It was anticipated that an increase in drainage tension would result in an increased seepage rate. The explanation of the only partial satisfaction of that anticipation probably lies in the differences, already mentioned, which existed between the two soil columns. Column A, it will be noted, more than doubled its seepage rate when the drainage tension

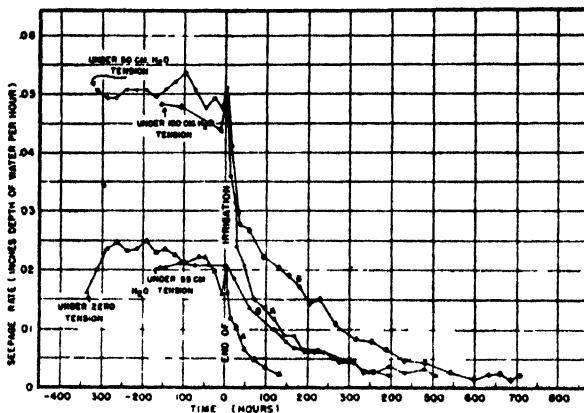


FIG. 2. DRAINAGE RATES, DURING AND AFTER IRRIGATION, OF SOIL COLUMNS HELD UNDER FOUR VALUES OF DRAINAGE TENSION

was increased from 0 to 50 cm. Likewise column B more than doubled its rate with an increase from 55 to 160 cm. When columns A and B were drained under nearly equal tensions, however, the seepage rate of column B was less than half that of column A, indicating differences in soil permeability between the two columns. From a study of figure 3 it may be inferred that the greater part of the permeability difference is found in the lower part of the two columns. It will be noted that during irrigation the pressure potential distributions are different in soil columns A and B although the individuals in each pair agree well. When these measurements were made, water was flowing through the columns and was not being absorbed at any level. Permeabilities are inversely related (under this steady-state condition) to the sum of the pressure potential gradients, indicated by the slope of the pressure potential lines, and to the gravitational potential gradient, which remains constant. Thus at the 20-inch depth in column A the pressure potential gradient is higher, and therefore the permeability is lower, than at the 40-inch depth, while here in turn the permea-

bility is lower than at the 65-inch depth. It will be observed that near the base of column B the permeability is lower than in the rest of the soil, whereas in column A it is higher at this level. It is possible that this, coupled with the generally higher pressure potential values in column B, indicate a somewhat lower permeability in this soil than in the other. A further interesting bit of evidence in favor of this is offered by the high potential differences between the lowest tensiometer and the bottom surface of the porous clay seepage plate during this period. Column A, under 50 cm. tension shows a drop from -16

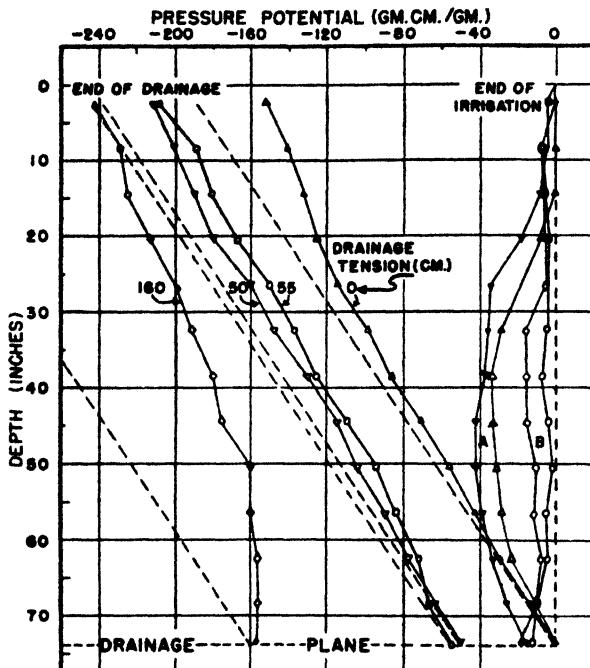


FIG. 3. PRESSURE-POTENTIAL CONDITIONS IN SOIL COLUMNS UNDER IRRIGATION AND AT THE END OF THE DRAINAGE PERIOD IN RELATION TO THE DRAINAGE TENSION EMPLOYED

gm. cm./gm. to -50, a difference of 34 gm. cm./gm. Column B shows a potential difference of 43 gm. cm./gm. under 55 cm. tensions, whereas under 160 cm. tension the difference rises to 142. The higher potential gradients exhibited by column B within this thin soil layer attest to its lower permeability, and therefore may serve to explain the differences in seepage rates observed during the irrigation period.

Chronologically, then, the first result of the application of moisture tension to the seepage plane of this tube has been to raise significantly the rate of seepage under irrigation. It has been shown, further, that raising the drainage tension from 0 to 50 cm. of water about doubles the seepage rate from this 6-foot column, and that the rate is again roughly doubled by raising the tension from 50 to 160 cm.

*Influence of drainage tension on soil moisture conditions during irrigation period*

Two of the sets of graphs in figure 4 show the soil moisture distribution in relation to depth during the irrigation period. The first set shows the distribution at the start of seepage under the drainage tensions studied. The second set shows conditions just prior to the termination of irrigation. At the start of seepage, significant moisture differences occur only at the bases of the soil columns. The soil drained under zero tension shows moistures here somewhat higher than the others. The moistures elsewhere, however, vary from 21 to 27 per cent and bear no unique relationship to the seepage tension.

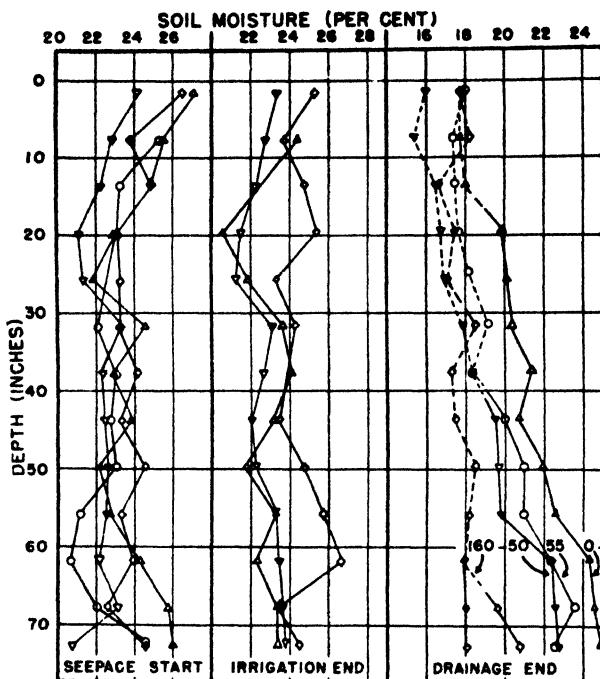


FIG. 4. SOIL MOISTURE CONDITIONS IN DRAINING AND DRAINED SOIL COLUMNS IN RELATION TO DRAINAGE TENSIONS  
Numbers refer to drainage tensions

At the end of irrigation the moisture distributions cannot be said to be closely related to the seepage tension applied. The general moisture range is again from 21 to 27 per cent and is not very different from that found at the initiation of seepage. The small range in pressure potentials shown in figure 3 at this time offers assurance that the moistures observed are true representations of soil conditions and have not been made inaccurate by difficulties often encountered in sampling very wet soil.

These moisture distributions demonstrate the persistent unsaturation of soil columns following prolonged irrigation and seepage, a condition which has been observed by Smith and Browning (7), Lauritzen *et al.* (4), and others.

The saturation moisture content of the soil used in this study is 27 per cent yet almost all moisture contents found at the end of the irrigation period were less than 26 per cent and the average was in the neighborhood of 23 per cent. Some authors ascribe the unsaturated condition to entrapped air. However, the negative pressure potentials which prevailed in these soil columns throughout the irrigation period (see fig. 3) indicate that moisture contents lower than saturation would be expected under the conditions of this study.

*Influence of drainage tension upon rate, total quantity, and period of drainage*

Referring again to figure 2, one will observe that, whereas during the irrigation period there were only two significantly different seepage rate levels, yet shortly after the cessation of irrigation the rate curve became significantly different in response to the drainage tensions imposed. At the end of 100 hours' drainage the seepage rates were approximately 0.003, 0.013, 0.011, and 0.022 surface inches per hour under 0, 50, 55, and 160 cm. tension respectively. At 200 hours the corresponding rates were 0.001, 0.006, 0.006, and 0.016. The same sequence of drainage rates was found at all times during the drainage period although

TABLE 1  
*Drainage of soil columns under four moisture tensions*

Moisture tension maintained during drainage.....	cm. $H_2O$	0	50	55	160
Period during which drain rate exceeded 0.002 inch/hour.....	days	6	17	21	30
Total quantity of drainage water (depth).....inches		1.1	4.3	4.0	7.8

the relative magnitudes varied greatly. The first influence of moisture tension upon drainage is thus shown definitely by the positions of the drainage rate curves.

The influence of drainage tension upon the duration of drainage and upon the total quantity of water removed from the soil is demonstrated in table 1. Under 50 cm. tension drainage continued at a rate in excess of 0.002 inch/hour almost three times as long as it did under gravity alone, and resulted in the loss of four times as much water from the soil. There is general agreement between this column and that drained under 55 cm. tension. A tension of 160 cm. quintupled the zero tension drainage time and produced over seven times as much water flow.

*Influence of drainage tension upon soil moisture conditions in drained soil*

One group of moisture:depth relationships in figure 4 shows the influence of drainage tension on the moisture remaining in the soil at the end of the drainage period. The drained moistures increase with depth, but all are below that representing pore space saturation. The significant differences between the three drainage conditions can best be demonstrated with reference to the 19 per cent field capacity of this soil. (Moistures greater than and less than 19

per cent are shown in the figure by solid and dashed lines, respectively.) The soil drained under gravity alone has moistures in excess of field capacity to a height of 57 inches above the seepage interface, leaving only a 17-inch thick layer of soil at or below normal drained field moisture. The soil drained under 50 cm. tension has moistures exceeding field capacity to a height of 33 inches, leaving a "normal wet" soil 41 inches deep. This agrees well with the soil drained under 55 cm. tension, which has moistures in excess of 19 per cent to a height of 34 inches.

In the soil drained under 160 cm. tension the abnormally wet soil layer is shown to be 8 inches thick, but evidence based on tensiometer records indicates that all the soil in this column is below its field capacity. Figure 3 presents

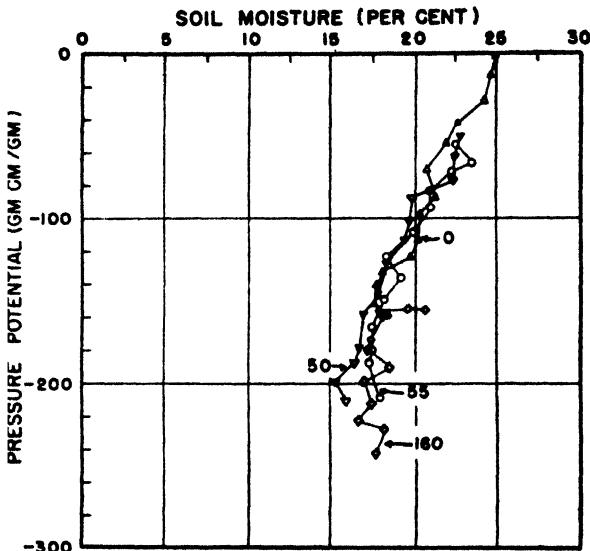


FIG. 5. RELATIONS BETWEEN SOIL MOISTURE AND PRESSURE POTENTIAL DETERMINED IN THE FOUR SOIL COLUMNS AT THE END OF THEIR DRAINAGE PERIODS  
Numbers represent drainage tension values

this evidence. Here it will be observed that the graphs representing the pressure potentials in the drained soil columns are significantly different and lie in a sequence such as would be expected in view of the magnitudes of the drainage tensions applied. Pressure potentials taken from this figure have been plotted in figure 5 in relation to the soil moisture values shown at corresponding depths in figure 4. The data for the four drainage conditions have been kept separate, and the points obtained from each connected in the order of increasing depth below the soil surface. The two lines representing the 0 and 50-cm. tensions show good agreement, but all the points obtained from the column drained under 160 cm. tension and those for lower moistures in the tube drained under 55 cm. fall to the right of the others, indicating that the soil used in the second tube filling (column B) was somewhat different from that in the first in its pressure-potential:moisture relationship. This may account for the somewhat

higher moistures found during the irrigation period in this soil column and for the fact that, in the upper half of the same column after the cessation of drainage, moistures were found equal to or higher than those in the column drained under 50 cm. tension. The upper end of the 160-cm. tension line in this figure suggests that the moistures determined in the lower part of this soil column were not representative of the true soil condition. The three upper points have equal pressure potential values although the moistures vary from 18 to 21 per cent. In the light of tensiometer records obtained during drainage and the moisture tension maintained at the base of this soil column, the pressure potentials appear to be correct. This leads one to the conclusion that the moistures at the two lower depths should be close to 18 per cent, rather than having the observed values of 20 and 21 per cent.

Increasing the drainage tension has thus been shown to result in a lowering of the average soil moistures in the drained soil. The response is shown most strikingly in the depth distribution of pressure potentials in figure 3. Here each pressure potential distribution is accompanied by a line representing zero total potential gradient under the drainage condition studied. This line is drawn at such a slope that the pressure potential decreases with height at the same rate as the gravitational potential increases. Since the gravitational potential increases 1 gm. cm./gm. (980 dynes) per centimeter increase in height, the equilibrium pressure potential is shown as decreasing at the same rate.

The small average total potential gradients operating in the soil columns at the end of drainage can be calculated from the data of figure 3. In the case of zero drainage tension the average pressure potential gradient between the base of the soil column and the uppermost tensiometer level is  $-0.83 \text{ gm./gm.}$  ( $\text{gm. cm./gm. per cm.}$ ). The algebraic sum of this value and the  $1 \text{ gm./gm.}$  value of the gravitational potential gradient results in a total potential gradient of  $0.17 \text{ gm./gm.}$  as the force responsible for downward water movement. In the soil columns drained under 50 and 55 cm. tension the average pressure potential gradient is  $-0.86 \text{ gm./gm.}$ , corresponding to a total potential gradient of  $0.14 \text{ gm./gm.}$ .

The pressure potential distribution in the column drained under 160 cm. tension departs considerably further from the equilibrium line than do the other two. The greater difference found here between equilibrium and observed pressure potential distributions may possibly be related to the low water permeabilities which prevail at moistures of less than field capacity, a relationship that has been demonstrated by Moore (5) and others. It has already been shown that at all levels in the drained column soil moistures were less than 19 per cent. It therefore appears probable that the pressure potential distribution in this column may still have been far from the equilibrium distribution by the time the drainage rate had dropped to such a low value as to be considered substantially equal to zero.

Figures 6, 7, 8, and 9 offer some clarification of the conditions leading up to the termination of drainage in the four soil columns. In each figure there have been plotted the pressure potential distributions determined in the soil at the

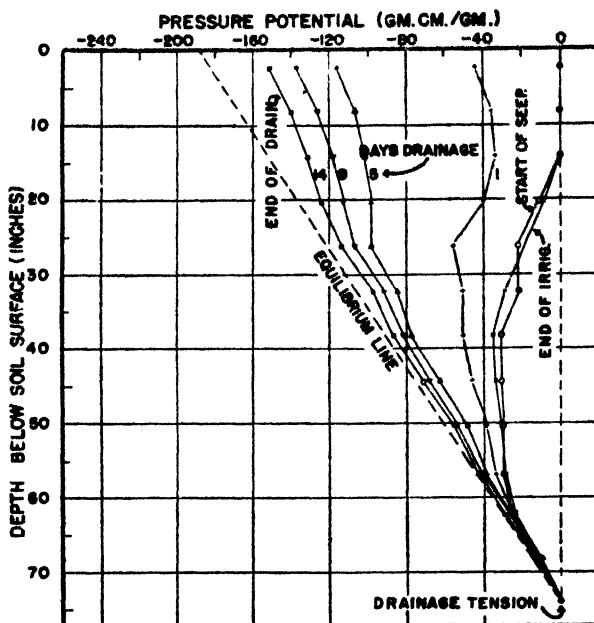


FIG. 6. PROGRESSIVE CHANGES IN THE RELATIONSHIP BETWEEN PRESSURE POTENTIAL AND DEPTH DURING DRAINAGE OF THE SOIL COLUMN DRAINED BY GRAVITY ALONE

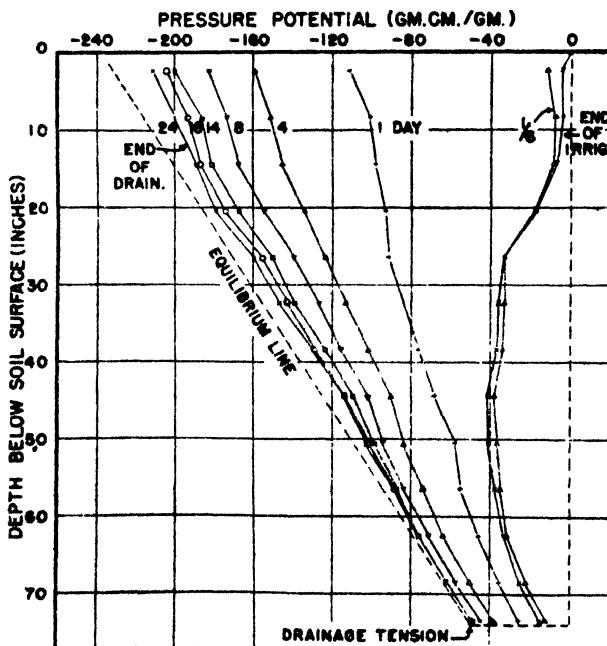


FIG. 7. PROGRESSIVE CHANGES IN THE RELATIONSHIP BETWEEN PRESSURE POTENTIAL AND DEPTH DURING DRAINAGE OF THE SOIL COLUMN IN WHICH THE POROUS DRAINAGE PLATE WAS HELD UNDER A TENSION OF 50 CM. OF WATER

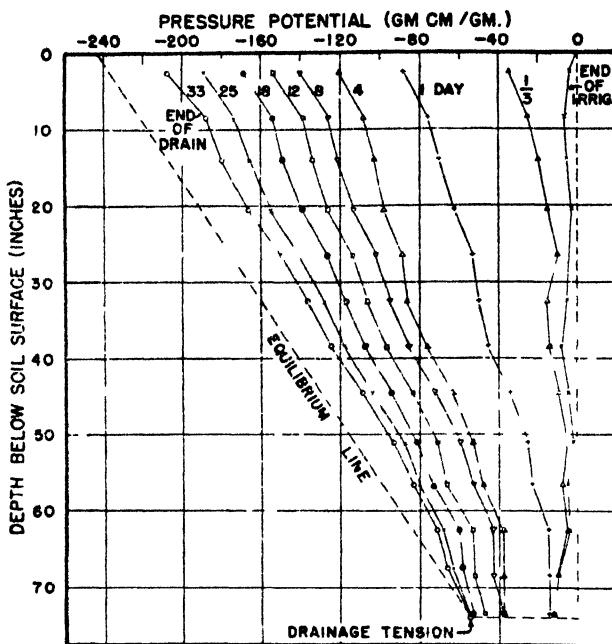


FIG. 8. PROGRESSIVE CHANGES IN THE RELATIONSHIP BETWEEN PRESSURE POTENTIAL AND DEPTH DURING DRAINAGE OF THE SOIL COLUMN IN WHICH THE POROUS DRAINAGE PLATE WAS HELD UNDER A TENSION OF 55 CM. OF WATER

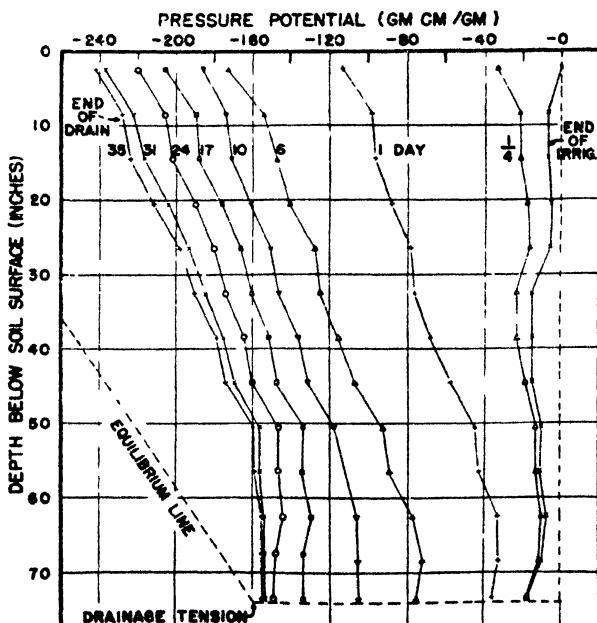


FIG. 9. PROGRESSIVE CHANGES IN THE RELATIONSHIP BETWEEN PRESSURE POTENTIAL AND DEPTH DURING DRAINAGE OF THE SOIL COLUMN IN WHICH THE POROUS DRAINAGE PLATE WAS HELD UNDER A TENSION OF 160 CM. OF WATER

start, during, and at the end of the drainage period. The four figures represent the four drainage tension conditions studied.

The pressure potential conditions in the soil before and after drainage have already been discussed with reference to figure 3. The conditions during the course of drainage are to be considered at this point. It will be noted that within one day after the cessation of irrigation the pressure potentials indicated in the uppermost tensiometers were considerably lower than those indicated in the lowest ones, showing the more rapid drainage of the upper than the lower portions of the soil column. In the soil drained under zero tension (fig. 6) the total potential gradient in the lower 12-inch layer of soil remained almost unchanged at a value very slightly in excess of zero throughout the whole drainage period. At each level above this layer the pressure potential decreased with time until it reached a value, on the fourteenth day, so close to the equilibrium line that water ceased to drain from the soil at a measurable rate. These considerations lead to the expectation, verified in figure 4, that the lower 12-inch layer served as a transmission zone for the drainage water, itself remaining at a constant water content while the water appearing as drainage has its source in decreasing moistures within the upper 5 feet of the soil column.

The situation is somewhat different in the columns drained under 50 and 55 cm. tension. As shown in figures 7 and 8 pressure potentials decreased continuously at all depths until the fourteenth and eighteenth days respectively, when the lowest tensiometer showed a pressure potential corresponding closely to the drainage tension. From this time until the end of drainage the lowest 12-inch layer remained under a total potential gradient very close to zero while the remainder of the column continued to lose water, albeit, (as shown in figure 2) at a much reduced rate.

Much the same progression of pressure potentials is shown in figure 9 for drainage under 160 cm. tension. Presumably because of low permeability, the drainage rate became immeasurably small, however, at about the time (31 days) when the pressure potential near the base of the soil column attained a value corresponding closely to the drainage tension applied. In this case there appears to be no time during the drainage period when any part of the soil column acted solely as a transmission zone, as was the case in the other two columns. It seems, rather, that water was being lost continuously from all depths but that the rate of loss decreased with time.

The porous clay plate at the base of the soil columns may, because of its low permeability, have presented a barrier to drainage. This may be concluded from a study of figures 7, 8, and 9 although figure 9 alone will be used to present the argument. The tension maintained below the clay plate may be expressed in the same units as the pressure potentials measured by the tensiometers within the body of the soil column. The drainage tension of 160 cm. of water thus corresponds to a pressure potential value of  $-160 \text{ gm. cm./gm.}$  Referring to figure 9, one will observe that during the greatest part of the drainage period a very high pressure potential difference was maintained between the lowest tensiometer level and the bottom surface of the clay plate, a distance of only

$\frac{1}{2}$  inch. This difference was not only greater than any observed in any other part of the soil column but was operative in the opposite direction, for here the pressure potential increased with height while through the rest of the column the reverse was true. As a result, the total potential gradient within the main body of the draining soil was less than unity whereas within the bottom  $\frac{1}{2}$ -inch layer it was much greater than unity. It is reasonable to assume that during the greater part of the drainage period the rate of water flow from the plane 6 inches above the soil base to the  $\frac{1}{2}$ -inch high plane was substantially equal to that from the  $\frac{1}{2}$ -inch height to the bottom side of the clay plate. If the permeability of the porous transmitting medium had been constant through this zone, the pressure potential gradient would likewise have been constant. Since the lower gradient was so much greater than the upper one, it appears likely that the permeability of the plate was markedly lower than that of the soil. This analysis suggests that the low permeability of the fired clay tension plate may have exerted a significant effect upon the drainage rate of the soil. The use of a fired clay plate of higher permeability would not have changed the moisture distribution in the drained soil, but it is probable that it would have brought about a more prompt drainage of the soil.

*Influence of drainage tension upon water content and air space in drained soil column*

In table 1 is shown the amount of water collected from the four 6-foot deep soil columns during drainage. It is of some interest to consider the amount of water left in the soil in relation to that present at the field capacity and at pore-space saturation. Also because of its influence upon the root development of lysimeter-grown plants, water-free pore space in the drained soils is worthy of study. Table 2 presents data covering these points.

The table shows that during the irrigation of the four soil columns air occupied only 4 to 7 per cent of the total soil volume, an amount considerably less than the 12.6 per cent calculated at field capacity, yet indicating some degree of unsaturation even under these conditions. As a measure of the lack of saturation, the 74 inches of soil contained between 26 and 29 inches of water, compared with a saturated water content of 31 inches.

At the end of the drainage periods significant differences had developed, both in average water content and in air space, in response to the drainage tensions employed. The soil drained by gravity alone still maintained a high water content (21.3 per cent average moisture and 24.5 inches depth of water), and its air space percentage (8.8 per cent) was considerably less than the field capacity value of 12.6 per cent. When the soil was drained under 50 and 55 cm. of water tension, the average moisture content, and therefore the average water content and air space percentage, were close to those at field capacity. As is shown in figure 4, however, this resulted from the averaging of moistures which varied from 16 to 23 per cent between the upper and lower ends of the soil column. The use of the average moisture and air space in this case would not result in the correct representation of the soil aeration conditions insofar as plant roots are

concerned, for actually the upper halves of these soil columns were well aerated while the lower halves were not. Soil moistures throughout the soil column drained under 160 cm. tension varied less than did those just discussed, which lends greater significance to the use of the average moisture value in this case. The average moisture and water content (18.1 per cent and 21.0 inches respectively) here are somewhat lower than those at field capacity and, correspondingly, the air space percentage is higher.

In terms of growing conditions for plants requiring normal field aeration of their roots, lysimeter drainage under some tension in the range from 50 to 160

TABLE 2  
*Summary of soil water status under four drainage conditions*

SOIL CONDITION	AVERAGE SOIL MOISTURE	AIR SPACE*	WATER CONTENT OF 74-INCH COLUMN
	per cent	per cent	inches
Water-saturated.....	27	0	31 -
Field capacity.....	19	12.6	22
Oven-dry.....	0	42.2	0
<i>At end of irrigation:</i>			
Drainage tension	0 cm..... 50 cm..... 55 cm..... 160 cm.....	23.9 22.8 23.1 24.6	6.9 6.6 6.2 3.8
			26.5 26.4 26.8 28.6
<i>Drained:</i>			
Drainage tension	0 cm..... 50 cm..... 55 cm..... 160 cm.....	21.4 19.0 19.7 18.1	8.8 12.6 11.5 14.0
			24.5 22.0 22.8 21.0

\* Air space is percentage of gross soil volume occupied neither by water nor by soil.

cm. of water appears more desirable than drainage under gravity alone. Comparisons of the average drained moistures show also that soils drained under these tensions have moistures approaching more closely to the natural field capacity values of the soil than do those drained under zero tension.

#### DISCUSSION

This study has demonstrated the possibility of removing the water in excess of field capacity usually found in enclosed lysimeter soils. Since lysimeters are intended to provide a soil and plant environment closely approximating that found in nature, the removal of excess water resulting from the presence of enclosing walls should improve the quality of the results obtained from them.

This study has shown that the rate of seepage is increased by the application of tension at the drainage plane but that the permeability of the clay plates used to maintain tension may exert a strong influence upon the rate and duration of drainage. The solution of the problem thus presented may lie in the use

of a large absorbing surface or of clay plates of high porosity, which, however, have pores sufficiently small that, when wet, air will not be drawn through them under the drainage tension used.

For the soil used in this study the proper drainage tension appears, from figure 5, to be about 125 cm. of water, which is equivalent to the pressure potential of -125 gm. cm./gm. at the 19 per cent (field capacity) moisture content. As shown by Moore (5) and in work now being conducted by the author, the pressure potential at the field capacity or moisture equivalent depends upon the moisture content representing these values, decreasing as the field capacity or moisture equivalent increases. This indicates that a lysimeter filled with sandy soil would require a lower drainage tension than would one filled with a soil of finer texture.

The use of drainage tension in lysimeters exposed under field conditions may entail difficult problems. Decisions as to kind, number, and placement of the tension devices will depend upon the characteristics of the installation and the kind of soil in the lysimeters. The water tension may be controlled by a simple "hanging water column" or may require some type of vacuum pump equipment. In order to study the practical problems involved in field installations, several 1600-pound weighing type lysimeters of the San Dimas installations have been equipped with drainage controls and the reaction of soil, water, and plants within them is now being compared with the same variables in "gravity drained" tanks of the same kind.

#### SUMMARY

A cylindrical column of soil 6 inches in diameter and 6 feet long was irrigated and drained four times, each time with a different moisture tension maintained at or beneath its base. Zero tension, corresponding to the usual gravity drainage of enclosed lysimeters, was effected by resting the soil on a perforated plate and collecting the drainage water that dripped from it. Drainage tensions of 50, 55, and 160 cm. of water were maintained on the lower surface of a porous fired-clay plate held in contact with the base of the column. A "hanging water column" for the 50 and 55-cm. tensions, and a vacuum pump for the other, provided the drainage tension. Moisture and pressure potential distributions were determined throughout the soil columns before, during, and after drainage; and measurements were made of rates and duration of drainage as well as of water outflow under each drainage tension.

The study showed that it was possible to control the seepage rate and the drained moisture content of a deep soil column by controlling the moisture tension maintained at the base of the soil. Analysis of pressure potentials within the soil during drainage indicated explanations of some of the drainage reactions observed.

On the basis of the present study, drainage of lysimeter soils under controlled moisture tension offers a possible solution to the problem of the removal of abnormal moisture usually found in those gravity-drained lysimeters in which seepage water must cross a soil/air interface.

REFERENCES

- (1) ADERKAS, O. J. 1930 Percolation of water into soil with various ground covers. *Mitteil. Forstl. Versuchs., Leningrad* 5: 41-51.
- (2) BODMAN, G. B. 1937 Variability of the permeability "constant" at low hydraulic gradients during saturated flow in soils. *Soil Sci. Soc. Amer. Proc.* 2: 45-53.
- (3) KITTREDGE, J. 1940, 1941 Report of the committee on evaporation and transpiration. *Trans. Amer. Geophys. Union* 1940 406-409; 1941 906-915.
- (4) LAURITZEN, C. W., AND STOLTENBERG, N. L. 1940 Some factors which influence infiltration and its measurement in Houston black clay. *Jour. Amer. Soc. Agron.* 32: 853-876.
- (5) MOORE, R. E. 1939 Water conduction from shallow water tables. *Hilgardia* 12: 383-426.
- (6) NEAL, O. R., RICHARDS, L. A., AND RUSSELL, M. B. 1937 Observations on moisture conditions in lysimeters. *Soil Sci. Soc. Amer. Proc.* 2: 35-44.
- (7) SMITH, R. M., AND BROWNING, D. R. 1942 Persistent water-unsaturation of natural soil in relation to various soil and plant factors. *Soil Sci. Soc. Amer. Proc.* 7: 114-119.
- (8) WALLIHAN, E. F. 1940 An improvement in lysimeter design. *Jour. Amer. Soc. Agron.* 32: 395-404.

# RELATION OF SOIL TYPE AND COMPOSITION TO THE OCCURRENCE OF NUTRITIONAL ANEMIA IN CATTLE

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A condition in cattle, locally termed "salt sick" (nutritional anemia), has been recognized by livestock owners to be related to the pasture area on which the animals are maintained. After being restricted for a time to certain pasture areas, animals decline in weight because of loss in condition and of reduction in amount of muscle tissue and in the volume of blood. To determine whether there is any relationship between soil type and composition and the occurrence of nutritional anemia in cattle pastured on the soils, a number of "healthy" and "sick" soils of Florida have been analyzed. This paper presents the results of the study.

## HISTORICAL

In 1888, Maxwell (11) noted similarity in some respects between "salt sick" and "southern cattle fever," based on the symptoms of a calf and an autopsy of it that he conducted at the Florida Agricultural Experiment Station. Stockbridge, French, and Ennis (18) assembled animals at a laboratory established to study "salt sick" in cattle where they conducted postmortem examinations and treatments of affected animals. Later, French<sup>1</sup> concluded that the condition was of nutritional origin, devised a mineral supplement with the aid of a pharmacist, and observed that young animals used more of the supplement than did older animals.

On somewhat brief contacts and observations, Dawson (7) noted some similarities to chronic tick fever, but finally (8) considered hookworms to be the causative agent. Both French and Dawson used forms of iron among their correctives.

In 1925, Shealy (17) reopened an investigation into the cause of "salt sick" and proved that feeds obtained from other areas would correct the condition. The investigation was expanded in 1929, with added personnel and facilities.

Iron and copper were pointed out as deficiencies in 1931 (2). These findings were based on field and controlled investigations with affected cattle. A supplement consisting of commercial grades of ferric ammonium citrate and copper sulfate and also a mineral mixture of common salt, ferric oxide, and copper sulfate were recommended and used to correct and prevent "salt sick," recognized as a nutritional anemia. The relation of cobalt to the condition was ascertained later (3, 15), following the discovery by Filmer and Underwood (9) that cobalt was essential to correct enzootic marasmus of cattle and sheep in West Australia.

Camp (6) pointed out the economic losses entailed in grazing cattle on marginal and deficient land. Separate technical papers have dealt with the hemoglobin content of the blood of anemic and healthy cattle (13), composition of feeds (14, 16) and of the soils (5) on which these feeds were grown, and reproduction in relation to mineral intake (1), general welfare of cattle (4), copper (12) and cobalt deficiency (3, 15) in relation to the complex problem of nutritional anemia.

## PROCEDURE FOR COBALT DETERMINATION

Normal hydrochloric acid was added to 100 gm. of soil in quantities varying from 50 ml. for very light sands to 400 ml. for soils containing higher concentrations of metals.

<sup>1</sup> French, W. E. Information to the senior author, 1929.

Variations of the quantity of acid used were considered to give a more nearly constant ratio of acid to soluble metals than would have been the case with a standard quantity of acid. It should be noted, however, that this cobalt analysis represented the acid-soluble cobalt under these conditions rather than total or available cobalt, and is of value mainly for comparison.

The soil and acid were allowed to stand for 18 hours with occasional stirring. The acid then was decanted and centrifuged to remove soil particles. An aliquot of this was digested with nitric and perchloric acid, and brought to dryness on a hot plate. The residue was taken up in  $N$  HCl. Sodium citrate was added and the pH was adjusted to approximately 8.6 with ammonium hydroxide. The solution was extracted with dithizone (diphenylthiocarbazone) and analyzed by a spectrographic internal standard method<sup>2</sup>.

#### RESULTS

The first group of soils<sup>3</sup> comprising numbers 15037 to 15180 were collected in 1930. Analyses of these soils for iron, copper, phosphorus, and acidity were published earlier (5). Certain of these analyses are republished in table 1 for comparison. Some of the original samples no longer were available at the time of the cobalt analysis, and hence are omitted.

Table 1 indicates a generally lower level of cobalt, iron, and copper for "sick" areas. Phosphorus has no consistent correlation with this condition. The italicized figures represent those that are below the apparent dividing level between "healthy" and "sick" soils, as classified according to reaction of cattle on the areas. It appears that some of the "sick" soils may contain sufficient copper. Whether or not 2.42 p.p.m. of copper should be used to mark the lower limits of copper-adequate range was questioned, since healthy dairy cattle on this land were fed heavily on concentrates grown outside the region. Hence the next higher soil in copper has been accepted as nearer to the lower limit for a "healthy" area. Soils below this limit were indicated as deficient in copper. It will be noted that the only "sick" soil which has an acid-soluble cobalt content of more than 0.020 p.p.m. has an unusually high pH value, 6.76. This factor may interfere with the availability of cobalt to the pasture plants.

The only three soil series that overlapped were Norfolk, Hernando, and Lakewood. One of the "sick" soils was Norfolk fine sand, contrasted with the "healthy" soil—*Norfolk fine sandy loam*. The Hernando, however, could be differentiated according to depth to clay subsoil, which was nearer to the surface with "healthy" soils. With these distinctions, the data suggest a good correlation of the cattle condition with the soil type. One Lakewood fine sand (No. 15137)

<sup>2</sup> Carrigan, R. A., and Erwin, T. C. Unpublished manuscript of a spectrographic internal standard method; to appear later.

<sup>3</sup> The soil samples in group 1 were obtained and the types identified in 1930 in cooperation with O. C. Bryan. These samples were from specific sites to correlate soil composition with nutritional condition of cattle. Later differentiations in soil classification (10) required that the original sites be reinspected in 1945 for verification or revision of the type names.

TABLE 1

*Composition of soils from "healthy" and deficient ranges, as related to the occurrence of nutritional anemia in cattle*

*Italicized figures represent values below an arbitrary level*

SAMPLE NO.	SOIL TYPE	ACID-SOLUBLE COBALT	COPPER	IRON	PHOS-PHORUS	pH
		p.p.m.*	p.p.m.	per cent	per cent	
<i>Soils from "healthy" ranges</i>						
<i>Group 1</i>						
15168	Archer fine sandy loam	1.300	6.46	0.768	.015	5.03
15172	Ruston fine sandy loam	0.300	9.36	1.445	.178†	5.10
15144	Ruston fine sandy loam	0.200	8.37	0.232	.005	4.84
15121	Bladen fine sandy loam	0.150	11.52	0.134	.003	5.19
15129	Archer loamy fine sand	0.121	4.21	0.203	.008	5.37
15133	Arredondo fine sandy loam	0.110	5.07	0.544	.070	6.19
15140	Norfolk fine sandy loam	0.110	7.39	0.555	.141†	5.33
15180	Ruston loamy fine sand	0.040	8.20	0.733	.156†	5.34
15137	Lakewood fine sand	0.035	2.42	0.077	.019	5.40
15152	Eulonia loamy fine sand	0.033	10.47	0.112	.005	5.54
15160	Hernando loamy fine sand	0.027	8.10	0.079	.009	4.87
15117	Bladen loamy fine sand	0.025	5.05	0.124	.008	5.00
15148	Matmon fine sandy loam	0.023	6.98	0.082	.005	4.93
Average.....		0.190	7.20	0.391	.050	....
<i>Soils from deficient ranges</i>						
<i>Group 1</i>		*				
15073	Davie fine sand	0.070	2.87	0.069	.007	6.76
15053	Blanton fine sand	0.020	4.51	0.058	.006	4.91
15093	Norfolk fine sand	0.016	1.50	0.086	.011	6.00
15081	Hernando fine sand	0.015	4.62	0.043	.010	5.68
15105	Hernando fine sand	0.011	5.01	0.065	.018	5.01
15049	Leon fine sand	0.007	1.61	0.007	.006	4.59
15061	Blanton fine sand	0.007	5.00	0.071	.004	5.28
15113	Norfolk fine sand	0.006	4.84	0.014	.015	5.62
15065	Portsmouth fine sand	0.006	7.85	0.023	.014	4.55
15109	Norfolk fine sand	0.005	5.26	0.061	.007	4.98
15045	Immokalee fine sand	0.005	2.01	0.018	.003	4.55
15101	Blanton fine sand	0.004	5.11	0.026	.009	4.85
15057	Leon fine sand	0.004	3.15	0.018	.005	4.93
15097	Blanton fine sand	0.004	4.49	0.041	.015	4.97
15037	Leon fine sand	0.003	1.96	0.014	.013	4.72
15077	Hernando loamy fine sand	0.003	3.42	0.054	.007	5.07
15089	Norfolk fine sand	0.001	2.81	0.055	.070	4.95
15085	Hernando fine sand	0.001	3.18	0.067	.030	5.46
Average.....		0.010	3.82	0.044	.014	....

\* p.p.m. = parts per million of soil.

† Three soil samples are from areas on which second growth trees or other features indicate possible cultivation at least 20 years prior to sampling of the soils. Barring these three samples, the average phosphorus contents were 0.017 and 0.014 per cent respectively.

containing 0.035 p.p.m. of acid-soluble cobalt and 2.42 of copper was on the dividing line as "healthy," whereas a similar soil in group 2 (No. 14064) had only 0.001 p.p.m. cobalt.

Two additional groups of soil samples, groups 2 and 3 (table 2), were collected in 1935 and 1937 from areas on which deficient cattle responded favorably to the use of cobalt supplement. Group 2 represented the pastures of 10 herds on six

TABLE 2

*Cobalt contents of two series of soils from areas on which livestock responded to cobalt therapy, following symptoms of deficiency*

SAMPLE NO.	SOIL TYPE	ACID-SOLUBLE COBALT p.p.m.*
<i>Group 2</i>		
14055	Leon fine sand	<.001
14060	Norfolk fine sand	<.001
14064	Lakewood fine sand	.001
14067	St. Johns fine sand	.008
14071	Portsmouth fine sand, mucky phase	.004
14074	Norfolk fine sand	<.001
14078	Leon fine sand	<.001
14081	Norfolk fine sand	<.001
14085	Immokalee fine sand	<.001
14088	Portsmouth fine sand	.002
Average.....		<.002
<i>Group 3</i>		
16887	Blanton fine sand	.004
16888	Blanton fine sand	.001
16889	Norfolk fine sand	.002
16890	Blanton fine sand	.003
16891	Blanton fine sand	.002
16892	Leon fine sand	.002
16893	Portsmouth fine sand	.007
16894	Leon fine sand	.002
Average.....		.003

\* p.p.m. = parts per million of soil.

soil types. Six of these soil samples contained less than 0.001 p.p.m. of acid-soluble cobalt, while the highest showed a content of 0.008 p.p.m. Group 3 represented four soil types ranging between 0.001 and 0.007 p.p.m. of acid-soluble cobalt. On two of these pasture areas, represented by samples 16888, 16889, and 16890, cattle had failed to respond to a mineral supplement of salt, iron oxide, and copper sulfate in addition to purchased concentrate feed. Addition of cobalt sulfate gave favorable and rapid recoveries of deficient animals on both pastures. The cobalt contents and soil types of groups 2 and 3 are presented in table 2.

Cattlemen recognized certain pasture areas to be "healthy" range, and prac-

ticed moving affected animals from the deficient ranges to these better areas, where recovery occurred. Three such pairs of instances are evidenced in the areas represented by the soil samples studied. The analyses of these soils are assembled for comparison in table 3.

The first pair of soils were taken from adjacent farms, between which a father moved his cattle from soil No. 15093 to his son's "healthy" pasture No. 15168, in which there was a substantial amount of Archer fine sandy loam. This interchange of the father's cattle was necessary about twice yearly, to keep the animals in thrifty condition. In the second pair, people in the community represented by soil No. 15089 hauled "salt sick" cattle to the "hospital farm" No. 15144, for

TABLE 3

*Comparison of soils from three sets of recognized "healthy" and near-by deficient ranges, between which cattle were moved because of recognized deficiency on the first-named area of each set*

SAMPLE NO.	SOIL TYPE	ACID-SOLUBLE COBALT p.p.m.*	COPPER p.p.m.	IRON per cent	PHOSPHORUS per cent	pH
15093	Norfolk fine sand	0.016	1.50	.086	.011	6.00
15168	Archer fine sandy loam	1.300	6.46	.768	.015	5.03
15089	Norfolk fine sand	0.001	2.81	.055	.070	4.95
15144	Ruston fine sandy loam	0.200	8.37	.232	.005	4.84
Group 3	Average of eight soils†	0.003	.....	.....	.....	.....
15121	Bladen fine sandy loam	0.150	11.52	.134	.003	5.19

\* p.p.m. = parts per million of soil.

† Average of eight soil samples representing Blanton, Leon, Norfolk, and Portsmouth fine sands which ranged between .001 and .007 p.p.m. of acid-soluble cobalt.

which they paid rent about a month or more. When the cattle recovered sufficiently they were led or driven back into the deficient area.

The fifth sample (No. 15121) was from a famous local range on which cattle recovered from "salt sick" rapidly. Cattle were moved from several miles around to this range, for the use of which there was keen competition. Cattle held too long on this range, however, developed a depraved appetite, evidenced by chewing bones, which is associated with phosphorus deficiency. This particular soil had a low phosphorus content despite its adequacy in other elements.

Recent observations of cattle on the three deficient areas (15093, 15089, and the third community) showed that the use of the recommended mineral supplement, which contained common salt, iron oxide, copper sulfate, and cobalt sulfate, ameliorated or prevented nutritional anemia and that the practice of "changing the range" for that purpose had been discontinued.

A number of other areas were encountered during the field phase of the nutritional investigation on which cattlemen followed the practice of "changing the range" to overcome the effects of deficiency incurred on certain ranges. In two instances, both the deficient and the "healthy" ranges were under the same owner-

TABLE 4  
*Distinguishing characteristics of the soil series enumerated in this report*

SOIL SERIES	COLOR		CONSISTENCE OF SUBSOIL	DEPTH OF SUBSOIL		PARENT MATERIALS	DRAINAGE	GREAT SOIL GROUP
	(A <sub>1</sub> horizon)	(A <sub>2</sub> horizon)		Fine sand	Loamy fine sand			
	brownish gray	yellow to brownish yellow	mellow, friable slightly compact	... 30-40 inches	15-30 inches	unconsolidated acid sands and clays	good	red and yellow podzolic
Ruston	gray	grayish yellow to yellow	mellow, friable slightly compact	40+ 30-40 ... 40+	15-30 30-40 ... ...	unconsolidated acid sands and clays	good	red and yellow podzolic
Norfolk	gray	Light gray or yellow and gray splotched	mellow, friable slightly compact	...	...	unconsolidated acid sands and clays	good to imperfect	red and yellow podzolic
Blanton	gray	yellowish gray to pale yellow	firm, friable, moderately compact	30-40 ...	...	unconsolidated acid sands and clays	good to imperfect	red and yellow podzolic
Eulonia	gray	dark brown to black	firm, friable, moderately compact	12-20 ...	...	unconsolidated acid sands and clays	imperfect to poor	ground-water podzol
Leon	light gray			,				

St. Johns	dark gray to black	light gray	dark brown to black	firm, friable, moderately compact	12-20	....	....	unconsolidated acid sands and clays	poor	poor	ground-water podzol
Immokalee	gray to dark gray	light gray	black to dark brown	mellow, friable, slightly compact	30-40	....	....	unconsolidated acid sands and clays	poor	poor	ground-water podzol
Portsmouth	dark gray to black	light gray	gray or gray and yellow mottled	mellow, friable, slightly compact	40+	....	....	unconsolidated acid sands and clays	poor	very poor	half-bog
Bladen	gray	light gray	gray or yellow and gray mottled	plastic, moderately compact	....	30-40	15-30	unconsolidated acid sands and clays	poor	poor	half-bog
Lakewood	light gray	white over yellow	....	....	....	....	....	unconsolidated acid sands and clays	excessive	dry sand	
Arredondo	brownish gray	yellow to brownish yellow	yellow to yellowish brown	firm, friable, moderately compact	....	....	15-30	phosphatic sands and clays	good	red and yellow podzolic	
Archer	gray	pale yellow to yellow	pale yellowish brown to orange	firm, friable, moderately compact	....	30-40	10-30	unconsolidated acid sands and clays over limestone	good	red and yellow podzolic	

TABLE 4—Concluded

SOIL SERIES	COLOR		CONSISTENCE OR SUBSOIL	DEPTH OF SUBSOIL		PARENT MATERIALS	DRAINAGE	GREAT SOIL GROUP
	(A horizon)	(B horizon)		Fine sand	Loamy fine sand			
Hernando	gray	pale yellow to yellow	firm, friable, moderately compact	40+	30-40	... soil clay sand lime	good	red and yellow podzolic
Davie	dark gray (peaty)	light gray	No B horizon, limestone substratum	24-60	... soil clay sand lime	... soil clay sand lime	poor to very poor	half-bog
Matmon	brownish gray	yellow to yellowish brown	plastic, moderately compact (thin over limestone)	... soil clay sand lime	12-24	... soil clay sand lime	imperfect to poor	lithosol

ship, and the "healthy" range was fenced apart so as to provide benefit for the maximum number of cattle from the more extensive deficient ranges.

A better understanding of the soils discussed in this report may be gained from table 4, which briefly summarizes the major characteristics.

It is believed that the condition of nutritional anemia in cattle may be associated with low availability of one or more of the elements cobalt, copper, and iron on certain the soil areas of this region. Further investigation is needed to establish the relationship between these three elements.

#### SUMMARY AND CONCLUSIONS

This report shows a definite correlation between low level of acid-soluble cobalt in the soil and the occurrence of nutritional anemia ("salt sick") in cattle, previously indicated as due to low iron and copper intakes.

The soil analyses suggest further that nutritional anemia may occur on soils where the limiting factor may be a low level of one or more of the elements cobalt, copper, and iron.

It appears both from soil analyses and from animal responses that nutritional anemia may be independent of the level of phosphorus in the soil.

It is believed, from the data presented, that it may be possible to predict whether or not an area may be "salt sick" by observing the soil type. Comprehensive analyses of soil samples may give further verification to any such prediction.

Use of the recommended mineral supplement has ameliorated or prevented nutritional anemia and made it unnecessary to "change the range" for that purpose. Mineral supplements intended for general use with cattle on these deficient areas well may contain all three elements—cobalt, copper, and iron.

#### REFERENCES

- (1) ARNOLD, P. T. D., NEAL, W. M., AND THOMAS, E. F. 1935 Reproduction in cattle in relation to mineral intake. *Assoc. South. Agr. Workers Proc. Ann. Conv.* 36: 525-526.
- (2) BECKER, R. B., NEAL, W. M., AND SHEALY, A. L. 1931 I. Salt sick: its cause and prevention; II. Mineral supplements for cattle. *Fla. Agr. Exp. Sta. Bul.* 231.
- (3) BECKER, R. B., AND GADDUM, L. W. 1937 The composition of limonite effective and ineffective in correcting "bush sickness" of cattle. *Jour. Dairy Sci.* 20: 737-739.
- (4) BECKER, R. B., AND HENDERSON, J. R. 1940 The welfare of cattle on Florida pastures. *Jour. Amer. Soc. Agron.* 32: 185-189.
- (5) BRYAN, O. C., AND BECKER, R. B. 1935 The mineral content of soil types as related to "salt sick" of cattle. *Jour. Amer. Soc. Agron.* 27: 120-127.
- (6) CAMP, P. D. 1932 A study of range cattle management in Alachua County. *Fla. Agr. Exp. Sta. Bul.* 248.
- (7) DAWSON, C. F. 1902 Texas cattle fever and salt-sick. *Fla. Agr. Exp. Sta. Bul.* 64.
- (8) DAWSON, C. F. 1906 Salt sick-bovine uncinariasis. *Fla. Agr. Exp. Sta. Bul.* 86.
- (9) FILMER, J. F., AND UNDERWOOD, E. J. 1934 Enzootic marasmus: treatment with limonite fractions. *Aust. Vet. Jour.* 10: 83-87.
- (10) HENDERSON, J. R. 1939 The soils of Florida. *Fla. Agr. Exp. Sta. Bul.* 334.
- (11) MAXWELL, G. T. 1888 Veterinary and physiology section. *Fla. Agr. Exp. Sta. Bul.* 2.

- (12) NEAL, W. M., BECKER, R. B., AND SHEALY, A. L. 1931 A natural copper deficiency in cattle. *Science* 74: 418-419.
- (13) NEAL, W. M., AND BECKER, R. B. 1933 The hemoglobin content of the blood of healthy and anemic "salt sick" cattle. *Jour. Agr. Res.* 46: 557-563.
- (14) NEAL, W. M., AND BECKER, R. B. 1933 The composition of feedstuffs in relation to nutritional anemia in cattle. *Jour. Agr. Res.* 47: 249-255.
- (15) NEAL, W. M., AND AHMANN, C. F. 1937 The essentiality of cobalt in bovine nutrition. *Jour. Dairy Sci.* 20: 741-753.
- (16) RUSOFF, L. L., ROGERS, L. H., AND GADDUM, L. W. 1937 Quantitative determination of copper and estimation of other trace elements by spectrographic methods in wire grasses from "salt sick" and healthy areas. *Jour. Agr. Res.* 55: 731-738.
- (17) SHEALY, A. L. 1926 Report of the veterinarian. *Fla. Agr. Exp. Sta. Ann. Rpt.* 1926: 85R-87R.
- (18) STOCKBRIDGE, H. E., FRENCH, W. E., AND ENNIS, J. E. 1901 Salt sickness. *Fla. Agr. Exp. Sta. Ann. Rpt.* 1901: 43-54.

# SOIL PROPERTIES AFFECTING THE PROPORTIONATE AMOUNTS OF CALCIUM, MAGNESIUM, AND POTASSIUM IN PLANTS AND IN HCl EXTRACTS<sup>1</sup>

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Although the mechanism by which mineral nutrients are mobilized into the growing plant is not fully known, evidence has accumulated showing that the release of metal cations from soil colloids is brought about by H ions, principally produced by the evolution of CO<sub>2</sub> from the roots. The employment of acids for measuring the release of cations is therefore a logical consequence. The various soil factors influencing the release of cations in equilibrium systems have been studied by employing electrolytes varying in their nature and concentration. Only limited attention has been given to the possible interrelationships of exchangeable Ca, Mg, and K; the chemical release of these cations by H ions; and their uptake by plants. In addition, the probable effects of total amounts of cations present, degree of saturation, cation ratios, and the type of colloid on the above aforementioned relationships have not been extensively studied. In this investigation the effects of cation-adsorption capacity, degree of saturation, cation ratios, and type of colloid on the interrelationship of the exchangeable Ca, Mg, and K and the proportionate amounts of these cations in plants and in the HCl extract of soils have been studied. The results are herewith presented.

The general literature pertaining to the interrelationship between Ca, Mg, and K uptake by plants and certain soil properties has been reviewed by Pierre and Bower (27), Peech and Bradfield (28), Beeson (4), Vandecaveye (32), and Schmalfuss (31). No simple relationship between exchangeable cations and their intake by plants is established. The relationship between exchangeable cations on colloid: sand mixtures and plant composition has been discussed by Albrecht (1). Van Itallie (14) investigated the effects of cation ratios in the soil on mineral composition of Italian rye grass. His results showed that the exchangeable bases in the soil could be directly related to the plant content, provided a specific factor was used to correct for the differential adsorption of bases.

The specific effects of cation competition have been studied by Jarusov (16) and by Jenny and Ayers (18). Jarusov concluded that where cations are directly assimilated by higher plants, lowering of saturation with respect to one ion decreases its availability. Furthermore, when given an identical degree of saturation, the availability of a cation is decreased by an accompanying ion having a lower energy of adsorption. Lowering the degree of saturation of K resulted in a reduced intake of that element by excised barley roots as reported by Jenny and Ayers. The rate of decline was greatly affected by the kind of the complementary ion. Ammonium had the greatest influence, calcium the least, and sodium and hydrogen occupied intermediate positions.

The release of cations by HCl, added at symmetry concentration, was reported by Giesecking and Jenny (8) and was in the slightly increasing order: Ca, Mg, and K. The order of

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release may be changed to a degree depending on the amount and kind of electrolyte used (8, 15, 17). The magnitude of cation release is greater from a freshly prepared colloid system than from one that has been aged (17). According to Elgabaly, Jenny, and Overstreet (7), a greater percentage of K was released from a K-clay system by adding HCl at symmetry concentration from colloids of the 1:1 lattice type than from the 2:1. Similar results were obtained by Allaway (2) and by Mehlich and Colwell (22) with respect to the release of Ca.

Bray (6) added 1.06 m.e. of H as HCl to soils which varied in cation-adsorption capacity from 9.9 to 37.9 m.e. per 100 gm. If his data are arranged in groups of somewhat similar adsorption capacities, the results show that with increasing percentage saturation the milliequivalents of Ca released increased but the percentage of Ca released decreased. The amounts of Ca, Mg, and K released are closely related to the distribution of the exchangeable cations in the soils. The "ease of release" is in the order Ca > Mg > K, that of Mg and K being somewhat greater in the groups of soils having the higher adsorption capacity. This tendency was predicted by the equations of Bray and is also in agreement with a theory advanced by Marshall (21).

#### MATERIALS AND METHODS

The source and preparation of most of the colloid materials used in this investigation have been reported elsewhere (22, 23) and are therefore given only brief mention here.

#### *Effect of H as HCl, added in amounts equivalent to the exchangeable Ca, on the release of Ca from different soils and colloids*

Several soils and materials were selected for this study; those representing the 2:1 lattice type were bentonite (Volclay), Putnam, and White Store. Volclay is a commercial product, the Putnam colloid came from the Missouri Agricultural Experiment Station, and the White Store soil came from a B<sub>2</sub> horizon in Chatham County, North Carolina. Representatives of the 1:1 lattice type used were "Kamec" kaolin, halloysite, and a subsoil sample of Durham. The Durham was obtained from the B<sub>1</sub> horizon of a soil near Raleigh, North Carolina. Hydrous mica-like colloids selected were illite and Collington subsoil (greensand). The organic system was represented by a peat obtained from Florida. Further details pertaining to the source of the above materials are reported elsewhere (23).

Colloid separates were obtained in the case of Durham, Collington, Putnam, and illite. The others were used without separation.

Hydrogen systems of these soils and materials were prepared and then Ca-soils or materials were prepared by adding Ca(OH)<sub>2</sub> to the H systems, aspirating with air, filtering, air-drying, and grinding to pass a 40-mesh sieve.

The release of Ca from these soil or mineral systems was effected by adding H as HCl in concentrations equivalent to the Ca in the sample and employing a colloid (or soil):solution ratio of 1:200 for peat, 1:100 for bentonite and Putnam, and 1:50 for the other soils and colloids. The total volume was 100 ml. in all cases. The suspensions were shaken for 15 minutes in a mechanical shaker and filtered, and the Ca was determined in a 75-ml. aliquot by precipitation as the oxalate and titration with permanganate.

*Release of Ca, Mg, and K by 1 m.e. H and the amounts of these cations in soybeans*

A study of the release of Ca, Mg, and K by 1 m.e. H was carried out with the same soil: sand mixtures used in a previously described investigation with cotton and soybeans (22). The natural contents of exchangeable bases of the soils used (White Store and Durham) were low. Calcium hydroxide was added to the soils in amounts to supply 20, 40, 60, and 80 per cent saturation with Ca. After a reaction period of about 2 months, the materials were dried, screened, and mixed with amounts of sand sufficient to obtain a cation-adsorption capacity of 4 m.e. The cations already present were taken into account, and Mg was added as the sulfate to supply a constant amount of 0.4 m.e. in one series and different levels (0.2, 0.4, 0.6, and 0.8 m.e.) in another series. In the same manner K was supplied as the monobasic phosphate and as the sulfate to give 0.2 m.e.; the amount of P supplied was 5 mgm.  $P_2O_5$  per 100 gm. Nitrogen at the rate of 5 mgm. per 100 gm. of soil was added as  $NH_4NO_3$ . These mixtures were used in 1942 in a greenhouse study with cotton which was allowed to grow for 40 days. After harvest the soils were allowed to air-dry. Replicate treatments were then composited and mixed thoroughly; samples were taken; and the cation-exchange properties and release of cations were determined by means of the  $BaCl_2$ -triethanolamine procedure (23). The soil:sand mixtures were again potted and seeded with soybeans. At this time a muck:sand mixture was included in the experiment. The nutrients were supplied as in the previous manner except that no N was added. The soybeans were grown for 54 days. The aerial parts were then harvested, air-dried, weighed, and analyzed for Ca, Mg, and K as previously described (22).

To study the chemical release of the cations 100 ml. of 0.002 *N* HCl was added to 20-gm. samples of the soil: sand mixtures; the suspensions were shaken for 15 minutes and filtered. The Ca was determined as above. The Mg was measured in the filtrate from Ca by a colorimetric procedure with titan yellow employing an electrophotometer. The K released was determined in a 75-ml. aliquot of a separate HCl extract by means of the nitroso-R-salt procedure as described under HCl extracts by Reed *et al.* (29).

*Effect of cation-adsorption capacity and degree of saturation on the uptake of Ca, Mg, and K by tobacco*

A White Store sample representing the 2:1 type of colloid was obtained from a B<sub>2</sub> horizon in Granville County, and a Cecil sample representing the 1:1 type of colloid was obtained from a B<sub>1</sub> horizon in Wake County, North Carolina. A muck sample was selected from the same location as that used for the study with soybeans. The two mineral soils were brought into a water suspension, and the colloid fraction was separated by decantation. The exchange properties were determined and  $Ca(OH)_2$  was added to obtain a 25 and a 50 per cent level of Ca. The materials were then mixed with sand to obtain cation-adsorption capacities of 3 and 6 m.e. per 100 gm. Magnesium was then added in solution as the

sulfate to give 0.25 m.e., and K as  $K_2HPO_4$  and  $K_2SO_4$  to give 0.125 m.e. per 100 gm. Tobacco was grown in these substrates in 1941. Samples for studying chemical release were no longer available, but the uptake of Ca, Mg, and K by tobacco was studied.

The analytical procedures for tobacco were the same as those described for soybeans (22). The determinations were made on the entire leaves only.

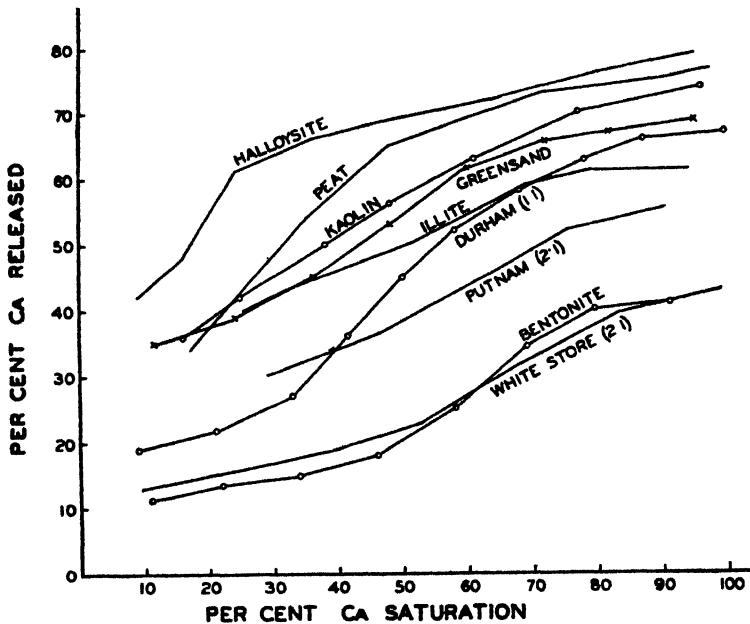


FIG. 1. RELEASE OF Ca BY HCl SUPPLIED IN AMOUNTS EQUIVALENT TO THE EXCHANGEABLE Ca AS INFLUENCED BY TYPE OF COLLOID AND DEGREE OF Ca SATURATION

#### RESULTS AND DISCUSSION

##### *Release of Ca as influenced by type of soil colloid and degree of saturation*

The percentage release of Ca by H, when supplied as HCl in amounts equivalent to the exchangeable Ca present, in relation to type of colloid and to degree of saturation is shown in figure 1. The data show the consistent increases in the release of Ca with increasing percentage saturation and the variations in release among different groups of base-exchange materials. The release is in the order of the organic > kaolinitic > hydrous mica > montmorillonitic groups. There are appreciable variations among the individual members within groups. The Ca release from halloysite is exceptionally high. Illite shows a relatively high release of Ca at the lower degrees of saturation but does not exceed 62 per cent at the higher levels of Ca. Although glauconite is included in the mica-like clay minerals, the sample used in this study (Collington) exhibits a higher release of Ca than illite.

These results with respect to the influence of degree of Ca saturation are in agreement with those of Allaway (2) and Parker and Pate (25). They are also in agreement with Allaway's results with respect to type of colloid. They are in the reverse order to that of the dissociation of H-clays calculated from activity measurements by Marshall (20), being montmorillonite > beidellite > hydrous mica > kaolinite. This order of release is not changed by either decreasing or increasing the amounts of H supplied at varying symmetry concentration levels, or when HCl is supplied in small but constant amounts.

The release of Ca by 1 m.e. of H supplied as HCl is shown in table 1. The milliequivalents of Ca released increase with increasing degree of Ca saturation.

TABLE 1

*Ca released by 1 m.e. H as HCl from soils and colloid:sand mixtures at varying levels of calcium saturation*

Ca SATURATION	BENTONITE*				KAOLIN*				MUCK*				PORTSMOUTH†				
	Cation-adsorption capacity, 4.8 m.e.		Cation-adsorption capacity, 2.4 m.e.		Cation-adsorption capacity 2.4 m.e.		Cation-adsorption capacity, 2.4 m.e.		Cation-adsorption capacity, 21 m.e.								
	Ca released				Ca saturation	Ca released	Ca saturation	Ca released	Ca saturation	Ca released	Ca saturation	Ca released	Ca saturation	Ca released	Ca saturation	Ca released	
per cent	m.e.	per cent	m.e.	per cent	per cent	m.e.	per cent	m.e.	per cent	m.e.	per cent	m.e.	per cent	m.e.	per cent	m.e.	per cent
28	.14	10	.12	18	16	0.23	59	14	0.21	62	3	0.23	46				
37	.21	12	.18	20	31	0.40	54	26	0.34	52	10	0.60	30				
57	.37	14	.29	21	55	0.64	48	50	0.57	50	14	0.80	27				
80	.65	17	.54	28	82	1.02	51	73	0.86	49	36	0.98	13				
99	.95	20	.88	37	99	1.10	46	98	1.04	44	67	1.23	9				

\* Colloid-sand mixtures used in an experiment with peanuts (24).

† Used in a study with lime described previously under Portsmouth B (23).

In a few systems at the highest levels of Ca the amounts released exceed the milliequivalents HCl (1 m.e. H) supplied. The percentage release of Ca increases in bentonite and decreases in the other systems.

The milliequivalents of Ca taken up by plants is generally known to increase with increasing degrees of Ca saturation, other factors being constant. The Ca uptake expressed as percentage of the exchangeable, however, has been found to decrease, increase, or remain little affected by increasing degree of Ca saturation. With Ca as the quantitative limiting factor for growth, percentage release may be expected to increase. In the presence of an ample supply of total Ca but limited with respect to its availability, the percentage uptake may be expected to vary with different crops and with the type of colloid. Thus, when plants were grown on White Store ranging from about 20 to 80 per cent Ca saturation, the Ca taken up by cotton increased from 0.4 to 1.3 per cent (22), and that taken up by soybeans remained virtually unaffected (see table 2). The plants grown on Durham having about the same range of Ca showed, however, a decrease in the uptake of Ca from 5.4 to 1.8 and from 7.8 to 5.1 per cent for cotton and soybeans, respectively.

According to Vageler-Alten [see Scheffer (30, pp. 96-99)] cation-absorption by plants is intimately related to the quantity of CO<sub>2</sub> produced by roots, which varies widely for different plant species [Scheffer (30, pp. 23, 98)]. Hence, for a given extent and character of root systems the percentage uptake of Ca may be expected to decrease either with increasing degrees of Ca saturation or with increasing cation-adsorption capacity at any given degree of Ca saturation. These trends are realized with HCl supplied in small but constant amounts. In systems of limited Ca supply as used by Allaway (2) or under conditions in which the extent of root distribution is governed by the degree of Ca saturation, the trends of percentage Ca uptake are probably more accurately represented by the addition of H ions equivalent to the exchangeable Ca present. The usefulness of these criteria will be more fully considered following the discussion of Ca, Mg, and K release by H and the relative uptake of these cations by plants.

*Relationships between the exchangeable Ca, Mg, and K in soil and their proportionate amounts in the HCl extract and in plants*

In predicting the proportionate amounts of cations displaced from soil by HCl, Bray (6) developed the equation which for Ca is as follows:

$$\text{Ca released} = \frac{\text{Ca}f_{\text{Ca}}}{\text{Ca}f_{\text{Ca}} + \text{Mg}f_{\text{Mg}} + \text{K}f_{\text{K}} + \text{H}f_{\text{H}} + \text{R}f_{\text{R}}} E$$

Where Ca, Mg, etc. = milliequivalents of exchangeable cations; f<sub>Ca</sub>, f<sub>Mg</sub>, etc. = constants for "ease of release"; and E = total cations released. The constants obtained under the conditions of his experiments were 1, 1.6, and 2.2 for Ca, Mg, and K, respectively. The constant for "ease of release" of calcium (f<sub>Ca</sub>) is "taken as 1.0 and the relative constants for potassium and magnesium are calculated by dividing the percentage release of each by the percentage release of calcium."

The values for the "ease of release" may be obtained from the quotients of the Ca:Mg or Ca:K ratios of the exchangeable cations in the soil divided by these ratios in the HCl extract. In applying this equation to some of these interrelationships involving plants, the values obtained from the quotients of these cation ratios in the soil and the Ca:Mg or Ca:K ratios in the plant (m.e. basis) are designated as cMg or cK as distinguished from fMg or fK obtained from HCl release data.

The relationships of the exchangeable Ca, Mg, and K in several soil:sand mixtures and the proportionate amounts of these cations in the HCl extract and in soybeans are shown by the results in table 2. The cation ratios of the soils, HCl extract, and soybeans, and the f and c values calculated from these ratios are given in table 3.

According to table 3 both the fMg and the cMg values are of somewhat the same order, whereas the cK values are very much greater than the fK values. Although there are a number of exceptions, both the f and c values tend to increase with increasing cation ratios in the soils. In several instances they approach a constant notably at the higher cation ratios. By maintaining the

Ca:Mg ratios approximately constant (treatments b and e), the effect of degree of Ca saturation may be ascertained. It is seen that for all three soil systems the fMg values are little influenced, and the cMg values are only slightly lower at about 80 per cent Ca saturation (treatment e) than they are at 40 per cent (treatment b).

TABLE 2

*Relationship of cations in soil, quantities released by 1 m.e. H, and quantities taken up by soybeans*

Cation-adsorption capacity of soil:sand mixtures 4 m.e. per 100 gm.

TREATMENT	CATIONS IN SOIL			CATIONS RELEASED BY HCl			CATIONS TAKEN UP BY SOYBEANS*								
	Ca		Mg	Ca		Mg	K		Ca		Mg	K			
	m.e.	m.e.	m.e.	m.e.	per cent	m.e.	per cent	m.e.	per cent	m.e.	per cent	m.e.	per cent		
<i>White Store:sand mixture (2:1 lattice type of colloid)</i>															
a	0.78	0.45	.198	0.25	32	0.29	64	.135	68	2.4	4.2	2.6	8.0	5.0	34.7
b	1.60	0.48	.195	0.38	24	0.27	56	.138	71	4.8	4.1	3.4	9.8	6.6	39.4
c	2.28	0.44	.202	0.50	22	0.21	48	.142	70	6.9	4.2	3.4	10.6	6.5	44.2
d	3.24	0.42	.205	0.63	19	0.15	36	.138	67	10.1	4.3	4.3	14.1	8.0	53.6
e	3.11	0.84	.195	0.58	19	0.38	45	.134	69	9.0	4.0	4.8	7.9	7.7	54.2
<i>Durham:sand mixture (1:1 lattice type of colloid)</i>															
a	0.74	0.36	.205	0.35	47	0.29	81	.162	79	4.2	7.8	2.1	8.0	5.8	38.9
b	1.52	0.37	.210	0.71	47	0.27	73	.166	79	7.7	6.8	2.9	10.8	7.2	47.4
c	2.29	0.37	.200	0.98	43	0.28	76	.180	90	8.9	5.4	2.6	9.7	8.3	57.2
d	3.12	0.35	.210	1.12	37	0.26	74	.175	83	11.5	5.1	2.6	10.2	7.2	47.3
e	3.10	0.69	.212	1.20	39	0.42	61	.168	79	9.5	4.2	2.9	6.0	6.8	44.1
<i>Muck:sand mixture (organic colloid)</i>															
a	0.80	0.35	.195	0.34	43	0.29	83	.170	87	5.0	8.6	3.4	13.4	8.1	57.0
b	1.46	0.35	.198	0.66	45	0.29	83	.182	92	8.6	8.1	3.8	14.9	8.4	58.3
c	2.24	0.36	.190	0.82	37	0.31	86	.185	97	9.3	5.2	3.9	14.9	8.8	63.7
d	3.18	0.35	.188	1.00	31	0.28	80	.178	85	10.7	4.6	3.2	12.6	10.3	75.0
e	3.12	0.76	.195	0.98	31	0.52	68	.172	88	8.2	3.6	3.3	6.0	9.0	63.4

\* The percentages of Ca, Mg, and K were calculated from the average of three replications on the basis of one pot, using the totals obtained by multiplying the m.e. of exchangeable cations by 72.6. The data pertaining to the contents of Ca, Mg, and K in soybeans expressed in m.e. per 100 gm. are reported elsewhere (22).

Increasing the amount of exchangeable Mg at a constant Ca:K ratio has shown a negligible effect on both the fK and cK values (compare treatments d and e). For any given treatment with respect to the cation ratios in soil the f and c values are highest in White Store and lowest in Durham; they are intermediate in muck with the exception of the cK values at the highest cation ratios.

The results shown in tables 2 and 3 were obtained with soil:sand mixtures having a cation-adsorption capacity of 4 m.e. per 100 gm. Soybeans were also

grown on the same soil:sand mixtures having a cation-adsorption capacity adjusted to 2 m.e. (22). This effect is revealed by comparing the average *f* and *c*

TABLE 3

*Cation ratios in soil, HCl extract, and soybeans and the quotients of the Ca:Mg or Ca:K ratios in soil divided by these ratios occurring in the HCl extract (fMg and fK) or in soybeans (cMg and cK)*

TREAT- MENT	Ca:Mg RATIOS			<i>f</i> Mg	<i>c</i> Mg	Ca:K RATIOS			<i>f</i> K	<i>c</i> K
	Soil	HCl	Plant			Soil	HCl	Plant		
<i>White Store</i>										
a	1.7	0.9	0.9	1.9	1.9	3.9	1.9	0.5	2.1	8.1
b	3.3	1.4	1.4	2.4	2.4	8.2	2.8	0.7	2.9	11.2
c	5.2	2.4	2.0	2.2	2.6	11.3	3.5	1.1	3.2	10.7
d	7.7	4.1	2.4	1.9	3.2	16.6	4.6	1.3	3.5	13.2
e	3.7	1.5	1.9	2.4	2.0	15.9	4.3	1.2	3.6	13.5
Average (cation - adsorption capacity 4 m.e.).....				2.2	2.4				3.1	11.3
Average (cation - adsorption capacity 2 m.e.).....				2.0	2.3				1.9	6.5
<i>Durham</i>										
a	2.1	1.2	2.0	1.7	1.1	3.6	2.2	0.7	1.7	5.1
b	4.1	2.6	2.7	1.6	1.5	7.2	4.3	1.1	1.7	6.7
c	6.2	3.5	3.4	1.8	1.8	11.4	5.4	1.1	2.1	10.6
d	8.9	4.3	4.4	2.1	2.0	14.8	6.4	1.6	2.3	9.3
e	4.5	2.9	3.3	1.6	1.4	14.6	7.1	1.4	2.0	10.4
Average (cation - adsorption capacity 4 m.e.).....				1.7	1.6				2.0	8.4
Average (cation - adsorption capacity 2 m.e.).....				1.4	2.0				1.7	7.6
<i>Muck</i>										
a	2.3	1.2	1.5	1.9	1.6	4.1	2.0	0.6	2.1	6.6
b	4.2	2.3	2.3	1.8	1.8	7.4	3.6	1.0	2.1	7.4
c	6.2	2.6	2.4	2.4	2.6	11.8	4.5	1.1	2.6	11.2
d	9.1	3.6	3.4	3.5	2.7	16.9	5.6	1.0	3.0	16.5
e	4.1	1.9	2.5	2.2	1.7	16.0	5.7	0.9	2.8	17.6
Average (cation - adsorption capacity 4 m.e.).....				2.2	2.1				2.5	11.9
Average (cation - adsorption capacity 2 m.e.).....				1.5	1.7				1.4	5.8

values for the cation-adsorption capacity at 4 and 2 m.e. shown in table 3. These results show the *f* and *c* values to be smaller for the 2 m.e. series with the exception of *cMg* in Durham. The magnitude of these differences is strongly influenced by the type of colloid.

The effects of cation-adsorption capacity and type of colloid on the cMg and cK values are further shown by the results with tobacco presented in table 4. In this case both cation ratios and total cations were held constant. Under these conditions the cK values were also consistently lower in the system having the lower cation-adsorption capacity, whereas the cMg values were little affected. With respect to type of colloid, the c values were higher in the soil of the 2:1 lattice type than in that of the 1:1.

The relatively good agreement between the fMg and cMg values is further substantiated by the results of Marshall (21). He found for both, release by

TABLE 4

*Effect of cation-adsorption capacity on the proportionate distribution of cations in tobacco leaves at constant cation levels and cation ratios in soil*

Cations in soil: Ca, 1.5; Mg, 0.25; K, 0.125 m.e. per 100 gm.

SOIL	CATION-ADSORPTION CAPACITY	CATIONS IN PLANT			cMg	cK
		Ca	Mg	K		
	m.e.	m.e.	m.e.	m.e.		
White Store (2:1)	3	143.2	50.5	76.8	2.1	6.3
	6	116.7	50.3	82.0	2.6	8.6
Cecil (1:1)	3	166.0	31.6	65.0	1.1	4.8
	6	153.9	31.5	82.0	1.2	6.3
Muck (organic)	3	214.5	35.2	86.4	1.0	4.8
	6	158.5	30.0	88.5	1.1	6.7

H ions and plant content, that the proportionate amounts of Mg to Ca were greater from the Lindley soil than from the Putnam soil. These relationships pertaining to the K-Ca systems were less consistent.\*

\* An examination of the data reported by Marshall reveals also the interesting fact that the relative release of Ca and Mn by HCl and the uptake of these cations by plants are apparently also governed by the same soil effects. The fMn and cMn coefficients calculated from some of his data and tabulated below illustrate the similarity of the soil effects for both release by HCl and uptake by plants. The values for both HCl extract and plants are invariably higher in Putnam. Increasing the Ca level in the soil increased the fMn and cMn values for Putnam; it had no effect on the coefficients of HCl and it had a slight effect on some of the plants for Lindley. The effect of substituting Mg in the soil is also essentially in the same direction for the Putnam soil but not in the Lindley soil, where these changes were small.

SOIL		PUTNAM SILT LOAM					LINDLEY SILT LOAM				
No.	Treatment	HCl	Blue grass	Redtop	Sweet clover	Korean lespedeza	HCl	Blue grass	Redtop	Sweet clover	Korean lespedeza
1	1 Ca	8.5	2.4	4.7	1.1	0.8	2.2	1.0	1.7	0.3	0.7
2	2 Ca	17.2	7.4	7.7	1.5	2.0	2.2	1.4	2.5	0.3	0.8
6	1 Ca, Mg	19.9	8.0	9.9	2.0	1.9	2.8	1.0	2.2	0.3	0.6
4	1 Ca, K	17.6	9.1	12.4	4.0	1.6	2.5	1.1	1.9	0.3	0.6

The *cMg* and *cK* values have been calculated also from different groups of data reported in the literature. In an investigation with lima beans, reported by Hester and Shelton (9), wide cation ratios of the soils were employed. The *cMg* and *cK* values calculated for the vegetative parts of these plants were  $1.95 \pm .25$  and  $6.0 \pm .75$ , respectively, when grown on Portsmouth (cation-adsorption capacity 27.4 m.e. per 100 gm.) and  $1.1 \pm .25$  and  $3.1 \pm .3$  when grown on Norfolk (cation-adsorption capacity 4.8 m.e.). The Ca:Mg ratios in Portsmouth ranged from 2.4 to 25.1 and in Norfolk from 1.2 to 6.2. The corresponding ranges in the Ca:K ratios were 1.8 to 35.4 and 4.7 to 12.7. Although the coefficients are relatively constant over a wide range of cation ratios the effect of type of soil is very pronounced.

In an experiment with alfalfa reported by Hunter, Toth, and Bear (12) the Ca:K ratios in the soil ranged from 1 to 32 before the first harvest and from 4 to 714 at the end of the seventh harvest. The *cK* values calculated from the first harvest increased with increasing cation ratios from 2.2 to 10.4; and were relatively constant at 8.2 when calculated from the seventh harvest, with the exception of those having the extreme Ca:K ratios (viz. 4 and 714). In another investigation with alfalfa (3) grown on widely different soils the range of the *cK* values was between 5.4 and 42.2 with the Ca:K ratios in soil varying between 6.4 and 101. The average *cK* value calculated for the first and the eighth harvest was 11.4 and 11.8, respectively. (For the seventh harvest the Ca and K removed by the plants were added to the milliequivalents of the exchangeable Ca and K found, before the *cK* values were calculated.) Roughly 60 per cent of the *cK* values were sufficiently close to the average to be considered a constant. A careful examination of the results revealed that soil differences had a greater effect on the *cK* values than did the Ca:K ratios, although some of the higher coefficients were found with the higher Ca:K ratios.

The effect of cation ratios on the *cMg* and *cK* values of plants grown in sand cultures may also be examined with interest. With identical Ca:K ratios (1 to 32) in soil and sand cultures, the *cK* values calculated from the data of Hunter (13) were of about the same magnitude for both series and increased from 2.1 to 10. The Ca:Mg ratios in the soil varied between 4.3 and 7.8 and in the sand between 1.25 to 2.4. The corresponding *cMg* values ranged from 2.2 to 2.4 and from 0.45 to 0.99 (the Mg value of 0.45 was obtained for only one system with the lowest Ca:Mg ratio, and the total range for the other treatments was from 0.84 to 0.99).

In the experiments thus far considered the calculations were based on the analysis of the entire top portions of a plant. The effect of cation ratios on the *cMg* and *cK* values calculated from the analysis of parts of plants may be observed from a consideration of data reported by Beeson *et al.* (5) and Hornberger (11). In the experiment of the former, tomatoes were grown in sand supplied with nutrients varying in their ratio but constant in their total amounts. The Ca:K and Ca:Mg ratios varied from 0.14 to 7.1. The *cK* values calculated from the chemical analysis of the tomato leaflets were 0.53 and 0.38 at the extreme cation ratios but varied only from 0.28 to 0.31 for the intermediate

Ca:K ratios. The cK values were but slightly influenced by the concentration of Mg. The cMg values, however, were notably influenced by K. Thus, when the Ca:Mg ratios varied from 0.25 to 3, the cMg values varied only from 0.66 to 0.77; they increased to 1.64, however, with the maximum concentration of K. The significance of these results lies in the fact that the cation interactions are manifest in parts of a plant, but, as in this case, the order of the proportionate amounts of cations contained in parts of the plant may be virtually the reverse from those of the over-all effect.

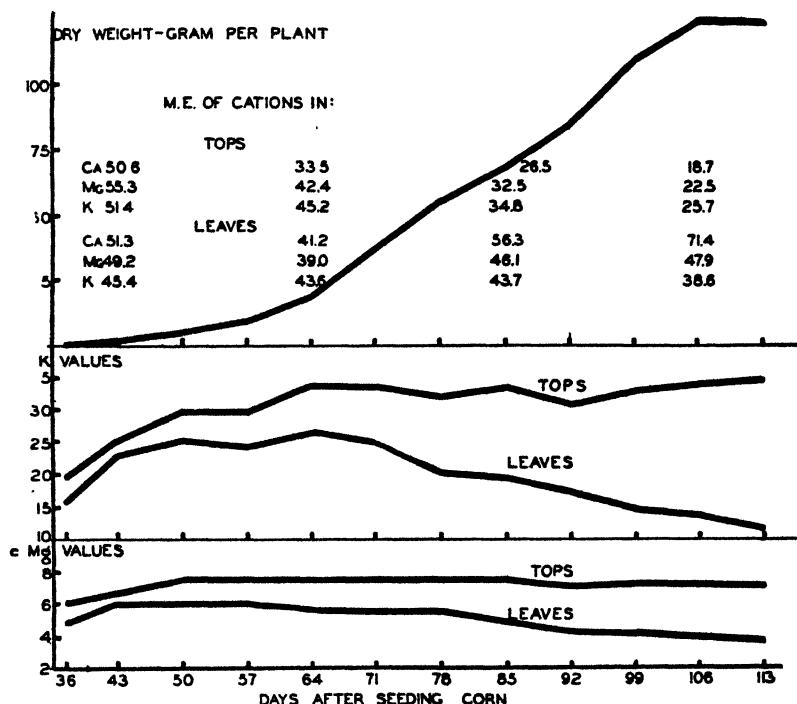


FIG. 2. VARIABILITY OF THE cMg AND cK VALUES OF LEAVES AND ENTIRE TOP PARTS OF CORN DURING A GROWTH PERIOD

A rather complete analysis of parts of the plants during a growth period has been reported by Hornberger (11) for corn. Since the exchangeable cations in this experiment were not determined, a Ca:Mg ratio of 6 and a Ca:K ratio of 25 were arbitrarily taken as representing the cation status of the soil before planting. These values were then used to calculate the cMg and cK coefficients from the analysis of the leaves and the entire tops of corn at weekly intervals. (It should be noted, therefore, that only the relative differences between parts of the plants and the variations due to stages of growth are pertinent.)

The results reported in figure 2 show that the coefficients are higher and more constant over a long period of growth for the entire top parts than for the leaves alone. The cK values from the analyses of the leaves decrease markedly after 71 days (tasseling period) and the cMg values after 78 days. The reason for

these differences may be verified by inspecting the results of the analysis shown for four periods at 3-week intervals in the upper part of figure 2. In the top parts of the plants the cation content decreases and after 64 days of growth they decrease in about the same ratio. In the leaves, the Ca and Mg first decrease and then increase, the ratio, however, becoming wider with the time of growth. The K content in the leaves remains relatively unchanged at first and decreases toward the last stages of growth. On the basis of the weight data of corn, also shown in figure 2, it appears that the consistency of the coefficients coincides with the period of rapid vegetative development.

TABLE 5

*Comparison of the determined and calculated Ca, Mg, and K content of red clover grown on soils with varying exchangeable cation levels*

Calculated from data of Lucas, Scarseth, and Sieling (19)

SOIL AND TREATMENT	DEGREE OF Ca SATURATION <i>per cent</i>	EXCHANGEABLE CATIONS IN SOIL			CATIONS IN RED CLOVER					
		Ca	Mg	K	Ca		Mg		K	
					Determined	Calculated	Determined	Calculated	Determined	Calculated
Bedford (NPK).....	49	5.2	0.60	.149	77.8	76.8	29.8	29.5	31.0	32.4
Bedford (LMNPK) .....	78	8.3	0.60	.186	78.8	88.8	28.8	21.4	32.8	30.0
Bedford (heavy fertilization).....	55	5.4	0.55	.286	61.0	61.0	22.4	20.3	46.5	48.6
Clermont (NPK).....	31	3.0	0.70	.138	56.0	47.8	26.8	36.7	35.2	33.5
Clermont (LNPK).....	64	6.4	1.08	.106	33.5	80.8	39.7	44.3	22.1	20.2
Crosby (Ck).....	56	8.7	2.50	.127	99.8	86.0	55.8	77.6	22.3	18.0

#### *Possibilities of predicting the proportionate amounts of Ca, Mg, and K in plants*

The proportionate amounts of Ca, Mg, and K in plants may be predicted by the equation of Bray when modified to provide the cMg and cK coefficient for plants. The degree of accuracy of this expression may be more fully ascertained by a consideration of the following experiment.

In a crop rotation experiment in the field on three soil types reported by Lucas, Scarseth, and Sieling (19) sufficient soil and plant analysis data were included to permit the calculation of the cMg and cK values. The average cMg coefficients were alfalfa 2.6, red clover 3.3, alsike clover 3.3, and mixed weeds 3.5; the corresponding cK values were 14.3, 15.0 21.0, and 32.3. In this experiment appreciable differences in the cation-exchange status of the soils were encountered. Using red clover as an example, the actual and calculated composition has been compared and the results are shown in table 5. The average coefficients ( $cMg = 3.3$ ,  $cK = 15$ ) calculated on the basis of ten treatments were used as constants. In table 5 the results of only six are given since they were representative of the range in soil differences. It will be noted that the agreement between the determined and calculated composition of all cations is satisfactory for the

plants grown on Bedford at 49 and 55 per cent and on Clermont at 64 per cent Ca saturation. For the crops grown on the other soils the cMg values deviated from the average to an extent rendering the comparison appreciably less accurate. It is thus evident that major trends in the distribution of the proportionate amounts of Ca, Mg, and K in plants may be predicted. For the attainment of greater accuracy, however, further modification of the equation, embodying the effects of degree of saturation, cation-adsorption capacity, and type of colloid, is essential. For this purpose the possibilities of supplementary criteria based on chemical release may be considered. This appears feasible inasmuch as several of the soil factors affect the uptake of Ca, Mg, and K by plants essentially in the same direction as those released by H ions.

The primary prerequisite for the use of these criteria is the establishment of the *c* values for different crops under conditions of known Ca release; this release to be measured by the addition of HCl in amounts equivalent to the exchangeable Ca present. Particular importance should be attached to that percentage release value above which a given crop no longer shows vegetative response. On the basis of the Ca-release value thus found for the lowest Ca level (degree of Ca saturation), the Ca:Mg and Ca:K ratios in the soil are varied so as to cover a wide range. The ranges of the *c* and *f* values are ascertained for both the crop and the HCl release obtained by adding a small but constant amount of HCl. The *c* and *f* values showing sufficient consistency are then averaged and are designated as constants for these standard conditions. The following expression for Ca, as an example, illustrates the factors considered:

$$\text{Ca in plant} = \frac{\text{Cac}_{\text{Ca}}}{\text{Cac}_{\text{Ca}} + \text{Mgc}_{\text{Mg}} \left( \frac{f_{\text{Mg}}^1}{f_{\text{Mg}}^0} \right) + \text{Kc}_K \left( \frac{f_K^1}{f_K^0} \right)} E$$

Where Ca, Mg, K = milliequivalents of exchangeable ions in soil; *c*Ca, *c*Mg, *c*K = constants obtained under standard conditions for a given crop calculated from the relationship Ca:Mg or Ca:K ratios in the soil/Ca:Mg or Ca:K ratios in the plant; *f*<sub>Mg</sub>, *f*<sub>K</sub> = constants for HCl release under standard conditions; *f*<sub>Mg</sub><sup>1</sup>, *f*<sub>K</sub><sup>1</sup> = values for HCl release of the unknown; and *E* = sum of the milliequivalents Ca, Mg, and K in plants.

The practical application of this approach to the prediction of the mineral composition of plants, based on the analysis of the exchangeable cations and percentage release under widely varying conditions, remains to be tested, since *c* and *f* values obtained from experiments conducted under "standard" conditions are not yet available. Nevertheless, a recognition of the effects of type of colloid on the release of cations may be helpful in explaining certain anomalous results.

Bray (6), for example, concluded, as predicted from his exchange equation, that "The substitution of calcium for hydrogen on a clay colloid, as is accomplished by liming, will decrease the relative release of the other ions, and, theoretically, make them less available to plant roots, *as far as the factors involved are concerned.*" Substitution of H for Ca will conversely increase the relative release of the other cations. These predictions are opposite to the conclusion of

Jenny and Ayers (18), who found the substitution of Ca for H to increase release of K; hence, liming renders K more available. The point is that both deductions could be correct in view of the type of colloids used in the experiments. Bray used the organic-beidellite-hydrous mica type, whereas Jenny and Ayers used systems of the montmorillonite type. But it is evident from the data in table 6 (calculated from some of the results in figure 1) that the coefficient for H is greater than 1 (when Ca = 1) for the colloids of the montmorillonitic type ( $fH$  1.4), and smaller than 1 for the other systems (illite  $fH$  0.58, peat  $fH$  0.3). Both conclusions, therefore, although apparently opposite, may be predicted from Ca-release data. The relatively good correlation of the data in table 6 obviously could not be obtained if an average  $fH$  value for all systems were used.

TABLE 6

*Comparison of the milliequivalents of Ca released with amounts of HCl equivalent to the exchangeable Ca and with those calculated\**

Ca IN COLLOID		Ca RELEASED		Ca IN COLLOID		Ca RELEASED		Ca IN PEAT		Ca RELEASED	
		Deter- mined	Calcu- lated			Deter- mined	Calcu- lated			Deter- mined	Calcu- lated
m.e.	per cent	m.e.	m.e.	m.e.	per cent	m.e.	m.e.	m.e.	per cent	m.e.	m.e.
Bentonite ( $fH = 1.4$ )											
19.3	22.3	2.62	2.66	6.41	32.4	2.73	2.25	41.0	16.9	14.0	15.8
29.4	34.0	4.35	5.74	10.21	51.6	5.20	4.74	86.0	35.4	46.1	47.0
50.2	58.1	12.50	14.70	13.68	69.1	8.04	7.36	144.1	59.3	100.7	95.6
59.8	69.2	20.70	19.75	15.65	79.1	9.60	9.04	174.0	71.6	127.6	124.0
78.3	90.6	32.20	30.80	18.50	93.5	11.35	11.40	219.2	90.2	164.5	165.5
Illite ( $fH = 0.58$ )											
Peat ( $fH = 0.3$ )											

\*After Bray (6) and modified with respect to  $D$ ,  $E = \frac{AC}{A(fa/fb) + C} D$  where  $E$  = total m.e. of cations released,  $A$  = m.e. of cations added,  $C$  = m.e. cation-adsorption capacity,  $D$  = degree of saturation, and  $fa, fb$  = average factor for  $A$  and  $C$  respectively.

The experimental material considered does not refute the general theory of the interdependence of the metabolic activity of roots and adsorption of cations (10, 28, 31). The magnitude of the  $c$  values for different crops grown under comparable soil conditions is probably due to differences in the metabolic activities of the roots (in terms of effective H ions produced). This, at any rate, may be implied from the results with HCl release. With amounts of H ions supplied to give 100 per cent release, an  $f$  value near to 1 may be expected. With ever-decreasing amounts of H ions supplied, the  $f$  values may be expected to increase ultimately to correspond to those obtained by activity measurements (21). The  $f$  value obtained with a given amount of HCl (1 m.e. H) represents only a single point on a curve of varying  $f$  values. It appears, therefore, that a single point thus chosen has little general validity in view of the fact that with different crops the  $c$  values are also different. Hence, the selection of several points on a curve of  $f$  values would seem to be more elucidative. Nevertheless, if the  $f$  values obtained from a determination of an arbitrarily selected single

point are used with reference to both *f* and *c* values obtained under standard conditions, its general application is probably enhanced, and more accurate prediction of the proportionate amounts of cations in plants are possible.

#### SUMMARY

The effects of soil colloids representing the organic, and the 2:1 and the 1:1 lattice type, cation-adsorption capacity, degree of saturation, and cation ratios on the interrelationship of the exchangeable Ca, Mg, and K and the proportionate amounts of these cations in plants and in the HCl extract of soil have been studied. The acid extract consisted of HCl supplied in small but constant amounts (1 m.e. H per 100 gm.) and in amount equivalent to the exchangeable Ca. The relationship of exchangeable Ca, Mg, and K and the proportionate amounts of these cations in plants included studies with soybeans and tobacco and a comparison with several other crops reported in the literature.

The proportionate amounts of the cations taken up by plants and released by HCl were in the order Ca > Mg > K. The proportionate amounts of Ca and Mg were of about the same order in both systems, whereas those of Ca and K in the plant systems were very much greater.

The quotients of the Ca:Mg or Ca:K ratios in soil divided by these ratios in the plant or HCl extract were influenced by varying soil properties as follows: (a) They increased with an increase in the cation adsorption capacity; (b) they increased with increasing degree of Ca saturation accompanied by an increase in the cation ratios of the soil; (c) they were relatively consistent within a limited range of Ca saturation but widely varying cation ratios of the soil, (d) they were little influenced by degree of Ca saturation when the cation ratios of the soil were held nearly constant, (e) finally, they were somewhat greater for the soils representing the 2:1 lattice type than for those representing the 1:1 lattice type, though this difference was small in the systems diluted with sand to give a low cation-adsorption capacity (2 m.e.).

Within a limited range of Ca saturation and similar types of colloid but widely varying cation ratios in soil, the quotients were sufficiently consistent to permit the prediction of the proportionate amounts of Ca, Mg, and K in plants. Supplementing the determination of the exchangeable ions by a limited release of these ions with small amounts of HCl increases the possibilities for predicting the proportionate amounts of these cations in plants under more widely varying conditions of soil properties.

#### REFERENCES

- (1) ALBRECHT, W. A. 1940 Adsorbed ions on the colloidal complex and plant nutrition. *Soil Sci. Soc. Amer. Proc.* 5: 8-16.
- (2) ALLAWAY, W. H. 1945 Availability of replaceable calcium from different types of colloids as affected by degree of calcium saturation. *Soil Sci.* 59: 207-217.
- (3) BEAR, F. E., AND PRINCE, A. L. 1945 Cation-equivalent constancy in alfalfa. *Jour. Amer. Soc. Agron.* 37: 217-222.
- (4) BEESON, K. C. 1941 The mineral composition of crops with particular reference to the soils in which they were grown. A review and compilation. U. S. Dept. Agr. Misc. Pub. 369.

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- (5) BEESON, K. C., LYON, C. B., AND BARRENTINE, M. W. 1944 Ionic absorption by tomato plants as correlated with variations in the composition of the nutrient medium. *Plant Physiol.* 19: 258-277.
- (6) BRAY, R. H. 1942 Ionic competition in base-exchange reactions. *Jour. Amer. Chem. Soc.* 64: 954-963.
- (7) ELGABALY, M. M., JENNY, H., AND OVERSTREET, R. 1943 Effect of type of clay mineral on the uptake of zinc and potassium by barley roots. *Soil Sci.* 55: 257-263.
- (8) GIESEKING, J. E., AND JENNY, H. 1936 Behavior of polyvalent cations in base exchange. *Soil Sci.* 42: 273-280.
- (9) HESTER, J. B., AND SHELTON, F. A. 1936 The influence of certain replaceable bases in the soil upon the elemental composition of vegetable crops. *Soil Sci.* 42: 335-340.
- (10) HOAGLAND, D. R. 1944 Lectures on the Inorganic Nutrition of Plants. Chronica Botanica Co., Waltham, Mass.
- (11) HORNEBERGER, R. 1882 Chemische Untersuchungen über das Wachsthum der Maispflanze. *Landw. Jahrb.* 11: 359-523.
- (12) HUNTER, A. S., TOTH, S. F., AND BEAR, F. E. 1943 Calcium-potassium ratios for alfalfa. *Soil Sci.* 55: 61-72.
- (13) HUNTER, A. S. 1943 A comparison of the response of alfalfa to identical Ca-K ratios in soil and in sand cultures. *Soil Sci.* 55: 361-369.
- (14) ITALLIE, TH. B. VAN. 1938 Cation equilibria in plants in relation to the soil. *Soil Sci.* 46: 175-186.
- (15) JARUSOV, S. S. 1937 On the mobility of exchangeable cations in the soil. *Soil Sci.* 43: 285-303.
- (16) JARUSOV, S. S. 1938 On the availability of soil exchangeable cations for plants. *Pedology* 33: 799-828.
- (17) JENNY, H. 1932 Studies on the mechanism of ionic exchange in colloidal aluminum silicates. *Jour. Phys. Chem.* 36: 2217-2258.
- (18) JENNY, H., AND AYERS, A. D. 1939 The influence of the degree of saturation of soil colloids on the nutrient intake by roots. *Soil Sci.* 48: 443-459.
- (19) LUCAS, R. E., SCARSETH, G. D., AND SIELING, O. H. 1942 Soil fertility level as it influences plant nutrient composition and consumption. Ind. (Purdue) Agr. Exp. Sta. Bul. 468.
- (20) MARSHALL, C. E. 1942 The use of membrane electrodes in the study of soils. *Soil Sci. Soc. Amer. Proc.* 7: 182-186.
- (21) MARSHALL, C. E. 1944 The exchangeable bases of two Missouri soils in relation to composition of four pasture species. Missouri Agr. Exp. Sta. Res. Bul. 385.
- (22) MEHLICH, A., AND COLWELL, W. E. 1943 Influence of nature of colloids and degree of base saturation on growth and nutrient uptake by cotton and soybeans. *Soil Sci. Soc. Amer. Proc.* 8: 179-184.
- (23) MEHLICH, A. 1945 Effect of type of soil colloid on cation-adsorption capacity, and exchangeable hydrogen and calcium as measured by different methods. *Soil Sci.* 60: 289-304.
- (24) MEHLICH, A., AND COLWELL, W. E. 1946 Absorption of calcium by peanuts from kaolin and bentonite at varying levels of calcium. *Soil Sci.* 61: 369-374.
- (25) PARKER, F. W., AND PATE, W. W. 1926 Exchange in soil colloids and the availability of exchangeable calcium in different soils. *Jour. Amer. Soc. Agron.* 18: 470-482.
- (26) PEECH, M., AND BRADFIELD, R. 1943 The effect of lime and magnesia on the soil potassium and on the absorption of potassium by plants. *Soil Sci.* 55: 37-48.
- (27) PIERRE, W. H., AND BOWER, C. A. 1943 Potassium absorption by plants as affected by cationic relationships. *Soil Sci.* 55: 23-36.
- (28) PREVOT, P., AND STEWARD, F. C. 1936 Salient features of the root system relative to the problem of salt absorption. *Plant Physiol.* 11: 509-534.

- (29) BREND, J. F., MEHLICH, A., AND PILAND, J. R. 1945 The use of nitroso-R-salt in the determination of exchangeable potassium in soils. *Soil Sci. Soc. Amer. Proc.* 9: 56-60.
- (30) SCHEFFER, F. 1938 *Agriculturnchemie, Teil B. Pflanzenernähr.* 35: 1-118.
- (31) SCHMALFUSS, K. 1936 *Das Kalium. Naturw. u. Landw.* 19: 1-98.
- (32) VANDECAYEYE, S. E. 1941 Effects of soil type and fertilization treatments on the chemical composition of certain forage and small grain crops. *Soil Sci. Soc. Amer. Proc.* 5: 107-119.



# FIXATION OF POTASSIUM IN RELATION TO EXCHANGE CAPACITY OF SOILS: I. RELEASE OF FIXED POTASSIUM<sup>1</sup>

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Since fixation of K in soils<sup>3</sup> is a widespread phenomenon, the problem of releasing the unavailable element is of economic importance as well as of academic interest. Accordingly, some investigations were undertaken in connection with a general study of K fixation.

Bartholomew and Janssen (2) found that some K unavailable at the start of a pot experiment was later utilized by certain plants. This does not necessarily mean that K was released from the "fixed" condition. Potassium is being released continually from minerals in soils, and this redction may differ markedly from the return to solution of K made insoluble by fixation.

Except in one soil, Hoagland and Martin (8), working with a variety of soils, found no results similar to those of Bartholomew and Janssen. They concluded that part of the K fixed by some soils in a nonreplaceable form will not maintain physiologically effective concentrations in the soil solution and is, therefore, of no practical value. The same investigators, in 1935 (9), added that fixed K cannot be released even by leaching with 0.2 N acid. Shaw and MacIntire (16) found that continuous extraction with ammonium acetate released no fixed K.

"The release of K from nonreplaceable forms during moist storage follows readily upon the removal of all or part of the original replaceable K," according to Bray and DeTurk (4). They reported further that release of K from non-replaceable forms under the same conditions (moist storage), but where no K has been added or removed, is generally negligible. Here again, however, conditions for the initial fixation of K were not well defined and the possibility of extraneous mineral sources of this element was not eliminated. The same objection is to be noted in the work of Allaway and Pierre (1), who found that they could release some fixed K by incubating soils under optimum conditions of temperature and moisture for 80 days. Blume and Purvis (3) viewed fixation as highly reversible. They asserted that K enters the fixed state, is released, and becomes fixed again in comparatively short time. They suggested further the possibility of a microbiological factor in fixation. Some of their experimental data are open to different interpretations. Kolodny<sup>4</sup> showed that K fixed in the soil was practically unavailable to plants.

<sup>1</sup> Part of a thesis submitted by the junior author in partial fulfilment of the requirements for the degree of doctor of philosophy in soil chemistry, 1942. Journal Series paper, New Jersey Agricultural Experiment Station, Rutgers University, department of soils.

<sup>2</sup> Temporarily withdrawn; resubmitted May 27, 1946.

<sup>3</sup> Fixation of K in soils is the conversion of soluble and replaceable K into a form which is not released by the cations generally used in exchange reactions.

<sup>4</sup> Kolodny, L. Mechanism of potassium fixation in soils and the availability of fixed potassium to plants. 1938. Unpublished doctor's thesis. (Copy on file, Rutgers University Library, New Brunswick, N. J.)

## EXPERIMENTAL

Previous work<sup>6</sup> on soil from the Dover series indicated that alternate freezing and thawing failed to release any fixed K. In another experiment it was found that even prolonged treatment on a steam bath with 0.05 N HCl released no K. This latter result led to the following experiment:

Hydrogen colloid ( $2\mu$  and smaller) was prepared from the B<sub>2</sub> horizon of a Dunellen profile and freed of organic matter by repeated treatment with H<sub>2</sub>O<sub>2</sub>. Of seventeen 10-gm. samples, twelve were treated with 30 ml. of standard KCl (1 ml. = 5.03 mgm. K) and five with water. The samples were wetted and dried five times at 110°C. to induce fixation. Two of the samples were then analyzed for fixed K, and the remainder were dispatched as follows: Separate

TABLE 1  
*Release of fixed potassium from Dunellen colloid*

INITIAL TREATMENT (PRIOR TO FIXATION)	SUBSEQUENT TREATMENT (ON STEAM BATH)	R <sub>2</sub> O <sub>2</sub> RELEASED	K INFILTRATE*	FIXED K RELEASED†
KCl	H <sub>2</sub> O	mgm.	mgm.	mgm.
	H <sub>2</sub> O	0.5	119.8	1.8
H <sub>2</sub> O	0.05 N HCl	32.5	121.2	0.8
	0.05 N HCl	33.4	5.3	...
KCl	0.1 N HCl	64.4	124.1	2.4
	0.1 N HCl	72.7	6.6	...
H <sub>2</sub> O	0.25 N HCl	148.2	125.8	4.1
	0.25 N HCl	157.0	6.6	...
KCl	0.5 N HCl	283.4	132.3	6.4
	0.5 N HCl	282.4	10.8	...

\* Without subsequent treatment, 115.1 mgm. K was found in filtrate. The Wilcox (21) method for determining K was used.

† 35.8 mgm. K was fixed in the initial treatment.

sets of two samples and one water blank were treated respectively with 200 ml of water, 0.05 N, 0.1 N, 0.25 N, and 0.5 N HCl. The samples were then placed on the steam bath for 72 hours. To avoid change in the concentrations of the various solutions, the operations were carried out in tall Erlenmeyer flasks fitted with long-stemmed funnels to serve as reflux condensers. When the samples were removed from the steam bath, they were thrown on filters and leached with water and then with neutral normal ammonium acetate. Sesquioxides and K were determined on the leachate. Results are shown in table 1.

<sup>6</sup> Levine, A. K. Relation of potassium fixation to the exchange capacity of soils. 1939. (Unpublished master's thesis. Copy on file, Rutgers University Library, New Brunswick, N. J.)

The data indicate that only very small quantities of K are released from the fixed state even by prolonged treatment with hot acid of considerable strength. There appears to be practically no release with acid up to tenth normal; at that concentration there is a small but steady and appreciable increase in K released with increasing acid concentration. No correlation is evident between K released and sesquioxides released.

To gain further insight into these results, the clay fraction ( $5\mu$  and smaller, organic-matter-free) from the B<sub>2</sub> horizon of a Collington soil was treated as follows: Fixation was induced as in the previous experiment in eighteen 5-gm. samples, ten treated with 20 ml. of standard KCl solution and eight with water. The K fixed by two of the samples was determined, and to separate sets of two K-fixed samples and two water blanks were added respectively 200 ml. of 0.5 N and 1.0 N HCl and 0.5 N and 1.0 N Na<sub>2</sub>CO<sub>3</sub>. Subsequent treatment and anal-

TABLE 2  
*Release of fixed potassium from Collington clay*

INITIAL TREATMENT (PRIOR TO FIXATION)	SUBSEQUENT TREATMENT (ON STEAM BATH)	R <sub>2</sub> O <sub>5</sub> RELEASED		FIXED K RELEASED†
		mgm.	K IN FILTRATE*	
KCl	0.5 N HCl	189.3	105.8	5.8
	0.5 N HCl	198.3	5.9	...
H <sub>2</sub> O	1.0 N HCl	271.2	122.5	5.7
	1.0 N HCl	271.7	22.7	...
KCl	0.5 N Na <sub>2</sub> CO <sub>3</sub>	4.8	111.7	0.0
	0.5 N Na <sub>2</sub> CO <sub>3</sub>	7.8	19.2	...
H <sub>2</sub> O	1.0 N Na <sub>2</sub> CO <sub>3</sub>	Not determined	180.5	0.0
	1.0 N Na <sub>2</sub> CO <sub>3</sub>	Not determined	39.6	...

\* Without subsequent treatment, 94.1 mgm. K was found in filtrate.

† 6.5 mgm. K was fixed in the initial treatment.

ysis were the same as in the previous experiment, except that water was omitted from the filter-washing. Results are shown in table 2.

Unlike the previous experiment, almost all the fixed K was released by the acid. The indications are that drastic action by relatively concentrated acid is required to put fixed K back into solution.

The results with the Na<sub>2</sub>CO<sub>3</sub> solutions are more striking. No fixed K was released by treatment with alkali even of normal concentration, and scarcely any sesquioxides were released. (Sesquioxides released by 1.0 N Na<sub>2</sub>CO<sub>3</sub> were not determined because of the small quantity of hydroxides precipitated by NH<sub>4</sub>OH.) The HCl treatment probably caused more mineral breakdown than the Na<sub>2</sub>CO<sub>3</sub>. This was borne out by an experiment in which it was found that the water blank treated with hot 1.0 N HCl would not subsequently fix K, whereas those treated with Na<sub>2</sub>CO<sub>3</sub> did.

A third experiment of a similar nature was made on Wyoming bentonite, chosen because of its marked ability to fix K and its low total K content. To sixteen 5-gm. samples, 25 ml. of KCl solution (1 ml. = 2.65 mgm. K) was added; to sixteen others, only water was added. Fixation was induced as before. Two of the K-fixed samples and two water blanks were analyzed for K fixation. The remainder were treated as in the previous experiment except that 0.05 N, 0.1 N, and 0.25 N HCl were also tested. Subsequent treatment and analysis were the same as before. The samples treated with HCl were washed on the filters with

TABLE 3  
*Release of fixed potassium from Wyoming bentonite*

INITIAL TREATMENT (PRIOR TO FIXATION)	SUBSEQUENT TREATMENT (ON STEAM BATH)	R <sub>2</sub> O <sub>5</sub> RELEASED	K IN FILTRATE*	FIXED K RELEASED†
KCl	0.05 N HCl	mgm.	mgm.	mgm.
	0.05 N HCl	15.6	49.0	3.1
H <sub>2</sub> O	0.1 N HCl	mgm.	mgm.	....
	0.1 N HCl	15.0	0.0	....
KCl	0.25 N HCl	mgm.	mgm.	....
	0.25 N HCl	31.2	49.0	3.1
H <sub>2</sub> O	0.5 N HCl	mgm.	mgm.	....
	0.5 N HCl	35.4	0.0	....
KCl	0.25 N HCl	mgm.	mgm.	....
	0.25 N HCl	49.9	56.6	10.7
H <sub>2</sub> O	0.5 N HCl	mgm.	mgm.	....
	0.5 N HCl	49.0	0.0	....
KCl	0.5 N HCl	mgm.	mgm.	....
	0.5 N HCl	53.8	59.7	13.8
H <sub>2</sub> O	1 N HCl	mgm.	mgm.	....
	1 N HCl	53.7	0.0	....
KCl	1 N HCl	mgm.	mgm.	....
	1 N HCl	78.8	61.8	15.9
H <sub>2</sub> O	0.5 N Na <sub>2</sub> CO <sub>3</sub>	mgm.	mgm.	....
	0.5 N Na <sub>2</sub> CO <sub>3</sub>	trace	52.3	6.4
KCl	1 N Na <sub>2</sub> CO <sub>3</sub>	mgm.	mgm.	....
	1 N Na <sub>2</sub> CO <sub>3</sub>	trace	0.0	....
H <sub>2</sub> O	1 N Na <sub>2</sub> CO <sub>3</sub>	mgm.	mgm.	....
	1 N Na <sub>2</sub> CO <sub>3</sub>	trace	61.1	15.2

\* Without subsequent treatment, 45.9 mgm. K was found in filtrate.

† 20.3 mgm. K was fixed in the initial treatment.

0.05 N HCl; the samples treated with Na<sub>2</sub>CO<sub>3</sub> were washed with neutral normal ammonium acetate. Results appear in table 3.

The data again show an increase in the liberation of fixed K with increase in acid concentration. At normal concentrations almost all the fixed K was set free. From the amount of sesquioxides put into solution by the hot acids, it is logical to suppose that the increasing K release with acid concentration is intimately associated with mineral breakdown.

In contrast with the results on the Collington colloid (table 2), alkaline treatment released appreciable quantities of the K fixed by bentonite. This may be accounted for by the property of bentonite to swell after alkaline treatment.

Mattson (12) and others have shown that bentonite, when sodium-saturated, may swell to twenty times its original size. It is possible that the swelling allowed the large hydrated Na ions to enter the interlayer space and attack the fixed K. Collington clay, carrying the mineral glauconite, which belongs to the montmorillonitic group, does not swell so much as bentonite. This clay is not expected, therefore, to release the fixed K readily.

It is interesting to note that only very small amounts of sesquioxides were put into solution by the  $\text{Na}_2\text{CO}_3$ . In conjunction with the discussion on swelling, this fact suggests two comments. First, though K is fixed by mineral materials of the same family (layer minerals)<sup>6</sup> in all cases, the specific minerals that do the fixing may vary considerably. Hence, it may be possible that while one fixing mineral may be attacked by  $\text{Na}_2\text{CO}_3$ , another may not. Second, though the mineral is attacked by the alkaline solution, it is not broken down in the same sense as in the acid treatment. This is borne out by the inappreciable quantities of sesquioxides solubilized, by the increase in the exchange capacity, and by the fixation capacity following the alkaline treatment.

These three experiments warrant the conclusion that in the cases under consideration, fixed K is tightly held and resists the actions of ordinary solvents. It seems probable that the K liberated by strong acids is a by-product in the destruction of the mineral material. These results would tend to support those who contend that fixed K is not readily available for plant growth.

More information on this point can be gleaned from a series of pertinent experiments run at the same time as the three main experiments already discussed. With minor variations, the supplementary experiments are concerned with the refixing of K liberated through the agencies just described.

In the first of these experiments, three systems originating from the experiment on the Dunellen colloid (table 1), were employed: 1, the two K-fixed samples which had not been subsequently treated (i.e., no attempt had been made at release); 2, the K-fixed samples which had been treated with 0.5 N HCl on the steam bath and on which some release had been obtained; 3, the water blanks which had been treated with 0.5 N HCl on the steam bath. To each of these systems 20 ml. of KCl solution was added and samples were then subjected to alternate wetting and drying.

The second of these supplementary experiments was with the Collington clay used in the work presented in table 2. The following systems were employed: 1, the K-fixed sample which had received no subsequent treatment (i.e., no attempt had been made at release); 2, the K-fixed sample on which some release was obtained by treatment with 1.0 N HCl; 3, the water blank which had been treated with 1.0 N HCl; 4, the K-fixed sample which had been treated with 0.5 N  $\text{Na}_2\text{CO}_3$ ; 5, the water blank which had been treated with 0.5 N  $\text{Na}_2\text{CO}_3$ ; 6, the K-fixed sample which had been treated with 1.0 N  $\text{Na}_2\text{CO}_3$ ; 7, the water blank which had been treated with 1.0 N  $\text{Na}_2\text{CO}_3$ . The procedure for fixation was the same as in the other experiments. In the alkaline-treated samples, the exchange capacities subsequent to the treatment were determined.

<sup>6</sup> This will be discussed in a subsequent paper.

The results of these experiments are shown in tables 4 and 5.

The data from table 4 show that where no attempt was made to liberate any fixed K, little further fixation occurred. This is not unexpected, since under the conditions of the experiment less K was added than in the case of the original fixation. Hence the colloid, having fixed all the K it was capable of fixing at the original K concentration, exhibited little tendency to fix K at lower concentration.

Where some release was obtained, refixation occurred. Further, the quantity of K refixed was slightly lower, but of the same magnitude as the K liberated by the acid treatment; this is readily seen by subtracting row 1 from row 2 in table 4. Why the refixation should not have been even lower is not clear.

TABLE 4  
*Refixation of K with Dunellen colloid\**

DESCRIPTION	K RELEASED	K REFIXED
	mgm.	mgm.
Fixed; no subsequent treatment.....	...	1.9
Fixed; treated with 0.5 N HCl .....	6.4	7.8
Blank; treated with 0.5 N HCl.....	...	14.0

\* See table 1.

TABLE 5  
*Refixation of K with Collington clay*

DESCRIPTION	EXCHANGE CAPACITY AFTER TREATMENT	K RELEASED	K REFIXED
	mgm.	mgm.	mgm.
Fixed; no subsequent treatment.....	Not determined	...	0.0
Fixed; treated with 1.0 N HCl.....	Not determined	5.7	0.0
Blank; treated with 1.0 N HCl.....	Not determined	...	1.0
Fixed; treated with 0.5 N Na <sub>2</sub> CO <sub>3</sub> .....	1.50	0.0	1.6
Blank; treated with 0.5 N Na <sub>2</sub> CO <sub>3</sub> .....	1.75	...	7.7
Fixed; treated with 1.0 N Na <sub>2</sub> CO <sub>3</sub> .....	1.65	0.0	0.2
Blank; treated with 1.0 N Na <sub>2</sub> CO <sub>3</sub> .....	1.85	...	10.6

Nevertheless, it is of interest to learn that under some conditions it is possible to fix at least part of the K that has been freed from the tied-up condition.

The highest "refixation" occurred in the sample where there was no original fixation. The fixation was lower than that obtained originally (see footnote, table 1), but this was due to the acid treatment of the colloid (the destructive effect of acid treatment is reflected in the sharp decrease in exchange capacity which accompanies such treatment) and also to the lesser amount of K added. The important thing is that fixation here was markedly higher than where the colloid was treated identically save for the initial K fixation. This is in harmony with the theory that fixed K tends to block further fixation and that the fixing capacity of colloid minerals is constant for fixed conditions.

Table 5 bears out the results of table 4. Where no attempt was made to liberate fixed K, no further quantity was fixed. Though K was released by the action of hot normal acid, no refixation occurred; virtually none occurred in the water blank. The strong action of the acid results in a breakdown of the colloid and destroys its power to fix. If the mineral is broken down to a considerable degree, no refixation can be expected; if the action of the releasing agent is more moderate, some K may possibly be refixed. In this connection the work of Fraps and Fudge (6) is of value. They pointed out that not all minerals or soil colloids are destroyed to like degree by the same acid treatment. As a matter of fact, they noted a great difference in the different cases.

The exchange capacities of the  $\text{Na}_2\text{CO}_3$ -treated samples help explain the results obtained with these samples. Evidently the release of K by these alkaline solutions does not come as a by-product of mineral destruction. Or if there is mineral breakdown in this process, a building up takes place parallel with it. Breazeale and Magistad (5), Magistad (11), Mattson (13), and Van der Meulen (18) demonstrated early that alkaline treatment of soil colloids resulted in a buildup of the colloid material as reflected in the increase in exchange capacity. That fixation in the case of the water blanks was greater after alkaline treatment than the fixation prior to any treatment is explained by this increase in the exchange capacity; that is, since more K was able to enter the exchange complex, more was capable of being fixed. Volk (20) also noted an increase in the capacity to fix K subsequent to  $\text{Na}_2\text{CO}_3$  treatment. Naturally, very much less K was fixed by the samples which previously had fixed K.

It is worth noting that the exchange capacity of the fixed systems is lower than that of the nonfixed systems. This is attributable to the decrease in exchange capacity which accompanies fixation (10, 17).

The foregoing experiments showed that fixed K was not readily made soluble and, hence, was not expected to be readily available for plant growth. In this connection, the question arose of how readily available is the K from minerals. Although the rate of solution of mineral K is known to vary, closer investigation of the release of mineral K on a specific mineral was considered worth while.

Sericite was chosen for these investigations because of its high K content and because of the work of G. W. Volk (19). Volk found that sericite failed to fix K; if, however, he treated the sericite with carbonic acid over a period of time, the mineral did fix K. Though he advanced no explanation, the results suggested that perhaps the carbonic acid had released some K from the mineral and, hence, had opened the way for subsequent fixation. If this were the case, it would be strong evidence that K is fixed as part of a mineral compound rather than in some sort of loose undefined combination. It was decided to look further into this possibility.

A 25-gm. sample of the crushed (but not finely ground) mineral<sup>7</sup> was covered with water in an Erlenmeyer flask and a continuous stream of  $\text{CO}_2$  was bubbled into the system. After 30 days the system was thrown on a filter, washed, and

<sup>7</sup> The sericite used for this work came from Staley, North Carolina, and was obtained through the cooperation of the Bureau of Mines.

the filtrate analyzed for K. It was found that no K was released by this treatment.

The crushed sericite was then ground in a ball mill for several hours and treated as before. Again, no K was released beyond that made exchangeable by the grinding process (see table 6).\*

As no release of K could be obtained by continuous treatment with  $\text{CO}_2$ , the method of electrodialysis was tried. It has been shown by Mattson (14) that prolonged electrodialysis leads to a slow degradation of the mineral material under treatment. It was felt that perhaps by this gentle yet effective method of attacking the mineral, K could be freed without unduly destroying the sericite, that is, that perhaps something analogous to the "holes" described by Pauling (15) and Gruner (7) might be created and thus form an avenue for fixation of K.

A 20-gm. sample of ground sericite was, therefore, put into the central chamber of a Mattson cell and electrodialyzed between platinum electrodes for 15 days. The cathode solution was frequently drained and replaced by distilled water during this time to facilitate dialysis. When the process was completed, the catholyte was analyzed for K. To take into account the K released by grinding

TABLE 6  
*Electrodialysis of sericite*

	mgm.
K in cathode chamber.....	44.1
K exchanged on sericite.....	32.3
K released by electrodialysis.....	11.8
K fixed (electrodialyzed sample).....	14.2
K fixed (HCl leached sample).....	1.0
K fixed as result of previous electrodialysis.....	13.2

the mineral, a separate sample of sericite was washed with 0.05 N HCl and the K in the filtrate determined.

To the mineral taken from the Mattson cell after electrodialysis, 20 ml. of 0.1246 N KCl was added, and the system was alternately wetted and dried to induce fixation. As a check on the effect of electrodialysis on fixation, a sample of ground sericite was first leached with 0.05 N HCl, and then, after being washed with water to remove the acid, was treated for fixation in the usual manner. This check performed the same function as electrodialysis in removing the K from the exchange positions and leaving the mineral saturated with hydrogen. The difference was in the further action of electrodialysis as contrasted to simple washing with dilute acid, and this was what the check sought to emphasize.

The results of this experiment are presented in table 6.

Although the quantity of K freed by the continued action of electrodialysis was not very large, it is to be reckoned with. It is interesting that the quantity of K fixed following electrodialysis was virtually the same as that released by the

\* Fixation was attempted on the carbonic acid treated sericite, but the results were negative.

same means. Further, it is evident that the fixation in this case can be attributed only to the previous electrodialysis treatment, since substitution of hydrogen for the other ions (chiefly K) of the exchange complex by the use of dilute acid led to virtually no fixation.

On the other hand, in the experiment in which sericite was treated with carbon dioxide, no K was liberated and no subsequent fixation of K could be detected; in this case K release was followed by almost equivalent K fixation. Apparently this was no mere coincidence. It seems evident that the K released certainly came from the mineral as part of its structure. This suggests, in this instance, at least, and probably more generally, that K is fixed as part of the mineral structure. If this is so, we have a better picture of fixation and gain a much better insight into the mechanism of the process. This theory will be developed at some length in subsequent papers of this series.

#### REFERENCES

- (1) ALLAWAY, H., AND PIERRE, W. H. 1939 Availability, fixation, and liberation of potassium on high-lime soils. *Jour. Amer. Soc. Agron.* 31: 940-953.
- (2) BARTHOLEMEW, R. P., AND JANSSEN, G. 1931 Rate of adsorption of potassium by plants and its possible effect on the amount of potassium remaining in soils from applications of potassium fertilizer. *Ark. Agr. Exp. Sta. Bul.* 265.
- (3) BLUME, J. M., AND PURVIS, E. R. 1939 Fixation and release of applied potash on three coastal plain soils. *Jour. Amer. Soc. Agron.* 31: 857-868.
- (4) BRAY, R. H., AND DETURK, E. E. 1938 Release of potassium from nonreplaceable forms in Illinois soils. *Soil Sci. Soc. Amer. Proc.* 3: 101-106.
- (5) BREAZEALE, J. F., AND MAGISTAD, D. C. 1928 Base exchange in orthoclase. *Univ. Ariz. Tech. Bul.* 24.
- (6) FRAPS, G. S., AND FUDGE, J. F. 1935 Decomposition of base exchange compounds of soils by acids and its relation to the quantity of alumina and silica dissolved. *Jour. Amer. Soc. Agron.* 27: 446-455.
- (7) GRUNER, J. W. 1935 Structural relationship of glauconite and mica. *Amer. Mineral.* 20: 699-713.
- (8) HOAGLAND, D. R., AND MARTIN, J. C. 1933 Absorption of potassium by plants in relation to replaceable, nonreplaceable, and soil solution potassium. *Soil Sci.* 36: 1-32.
- (9) HOAGLAND, D. R., AND MARTIN, J. C. 1935 Absorption of potassium by plants and fixation by the soil in relation to certain methods for estimating available nutrients. *Trans. 3rd Internat. Cong. Soil Sci.* 1: 99-103.
- (10) JOFFE, J. S., AND KOLODNY, L. 1938 Effect of alternate drying and wetting on the base exchange complex with special reference to the behavior of the potassium ion. *Soil Sci. Soc. Amer. Proc.* 3: 107-111.
- (11) MAGISTAD, O. C. 1929 Use of artificial zeolites in studying base exchange phenomena. *Jour. Amer. Soc. Agron.* 21: 1045-1051.
- (12) MATTSON, S. 1929 Laws of soil colloidal behavior: I. *Soil Sci.* 28: 179-220.
- (13) MATTSON, S. 1931 Laws of soil colloidal behavior: V. Ion absorption and exchange. *Soil Sci.* 31: 311-331.
- (14) MATTSON, S. 1933 Laws of soil colloidal behavior: XI. Electrodialysis in relation to soil processes. *Soil Sci.* 36: 149-163.
- (15) PAULING, L. 1930 Structure of micas and related minerals. *Natl. Acad. Sci. Proc.* 16: 123-129.

- (16) SHAW, W. M., AND MACINTIRE, W. H. 1936 Relationship between water-soluble, replaceable, and fixed fractions of potash additions to soils. *Soil Sci. Soc. Amer. Proc.* 1: 143-148.
- (17) TRUOG, E., AND JONES, J. R. 1938 Fate of soluble potash applied to soils. *Indus. and Engin. Chem.* 30: 882-885.
- (18) VAN DER MEULEN, J. B. 1935 Relation between cation exchange with aluminum silicates and their crystal structure. *Rec. Trav. Chim. Pays-Bas.* 54: 107-113.
- (19) VOLK, G. W. 1938 The nature of potash fixation in soils. *Soil Sci.* 45: 263-276.
- (20) VOLK, N. J. 1934 Fixation of potash in difficultly available form in soils. *Soil Sci.* 37: 267-287.
- (21) WILCOX, L. V. 1937 Determination of potassium. *Indus. and Engin. Chem., Analyt. Ed.* 9: 136-138.

## BOOKS

*Annual Review of Biochemistry.* Volume 15. Edited by JAMES MURRAY LUCK, JAMES H. C. SMITH, AND HUBERT S. LORING. Annual Reviews, Inc., Stanford University, California, 1946. Pp. 687. Price \$5

This latest volume of the series contains a highly important set of papers on biological oxidations; nonoxidative enzymes; plant carbohydrates; chemistry of the lipids, proteins, amino acids, steroids, and hormones; the metabolism of carbohydrates, fats, proteins, and amino acids; vitamins; biochemistry of teeth; growth factors for microorganisms; photosynthesis; respiration of plants; biochemistry of yeast; bacterial metabolism; immunochemistry; organic insecticides; viruses; and inactivation and detoxication of pressor amines. The several papers include bibliographies of 74 to 445 references, the total number being 3,777. The importance of this annual contribution to the several fields of science is such that many workers in these fields will want copies for their personal use.

*The Agricultural Development of the Middle East.* By B. A. KEEN. Oxford University Press, London, 1946. Pp. 126, plates 15. Price 5/-.

This is a report of May, 1945, to the Director General Middle East Supply Center. It presents information gained and conclusions drawn as a result of a survey of the agriculture of Cypress, Palestine, Syria, Lebanon, Transjordan, Iraq, Persia, Egypt, The Sudan, Eritrea, Ethiopia, The Aden Protectorate, Tripolitania, and Cyrenaica. The several chapters deal with the obstacles to agricultural improvement, methods of raising agricultural standards, scientific and technical problems, and the supply and development of agricultural information and advice. The conclusions begin with the statement that "the peasant is imprisoned within the walls of his own agricultural system: year by year his numbers grow and the walls remain." It is pointed out that great irrigation schemes have provided large new fertile areas but "the population has rapidly bred up to them." Medical science has eliminated many diseases and saved many lives, but it has "intensified the pressure of population on the land." Thus these are "often only palliatives and the price for surmounting a current crisis is the probability of having to meet the same crisis, magnified, in the future." There is plenty of food for thought in this report.

*Geography of Latin America.* Revised edition. By FRED A. CARLSON. Prentice-Hall Inc., New York, 1944. Pp. 566, figs. 175. Price, \$6.

This is an exceptionally lucid discussion of the cultural heritage and political evolution of Mexico, Central America, South America, and the West Indies. Consideration is given to the people themselves, their land economy, and their industries, transportation, and commerce. Since much of the wealth of Latin America lies in the agricultural resources, a large part of the material has to do with soils, crops, and livestock. Emphasis is placed on natural conditions and

the extent to which they have promoted or retarded progress. Those who are concerned with obtaining an over-all view of the agricultural potentialities of the lands to the south of the United States will find the book of great interest and value.

*Land Classification for Land-Use Planning.* By G. V. JACKS. Imperial Bureau of Soil Science, Harpenden, England, 1946. Pp. 90, figs. 2. Price \$4.

This is technical communication 43 of the Imperial Bureau of Soil Science. It gives a thorough review of proposals that have been made by research workers in this field of study the world over. The material is exceptionally well presented and should be of great interest to all those who are concerned in any way with this problem. The contents include the following topics: types of land classification, land inventories, land and soil types, land-use classes, use of natural vegetation as an indicator of land quality, systems of land classification, and estimation of productivity. An excellent bibliography of 99 papers is appended.

*pH and Plants.* By JAMES SMALL. D. Van Nostrand Company, Inc., New York, 1946. Pp. 216, figs. 16. Price \$4.

The subtitle reads "a book for beginners," but all those who have to do with soil-plant relationships will find this a very useful volume for reference purposes. The several chapters deal with the pH scale, buffers, carbon dioxide effects, and pH and plant sap, cell walls, protoplasts, enzymes, aquatic life, plants, plant pathology, succulents, and cytoplasmic life. A very useful bibliography of some 275 papers is appended. The book presents the most complete picture of this subject as related to plants that has come to our attention.

*Scientific Instruments.* By HERBERT J. COOPER. Chemical Publishing Co., Inc., Brooklyn, New York, 1946. Pp. 305, figs. 190. Price \$6.

The range of instruments now being used in physical measurements is such that there has been great need for a book to explain the principles on which such instruments are based and the conditions of their use. That is the purpose of this book. It is divided into five sections: optical instruments, including lenses, cameras, microscopes, polarimeters, photometers, range finders, refractometers, spectroscopes, and telescopes; measuring instruments, including density, dimension, electrical, fluid, pressure, temperature, time, speed, and weight; navigation and surveying, including barometers, gyroscopic instruments, compasses, sextants, and sounding and surveying instruments; liquid testing, with special reference to viscosity measurement; and miscellaneous instruments, including those for acoustics, calculations, and hardness, vacuum tubes, and thermionic valves. An impressive array of instrument manufacturers and individual specialists contributed to the volume. This is a very helpful book to have about.

THE EDITORS.

# ANALYSES OF SOME SIAMESE LATERITES<sup>1</sup>

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Bangkok, Siam

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More and more clearly is it evident to those who have to do with soil and other agricultural problems in equatorial regions that the generalizations as to the nature and characteristics of "tropical" soils, and especially as to the character of "laterite" and "lateritic" soils, which are found in leading works on soils (5; 6, pp. 366-418; 19, 21, 22; 24; 25, pp. 161-169) are not only inadequate but seriously misleading. It is not to be wondered at that such is the case, for the data that have been available for the preparation of general discussions dealing with equatorial soils leave much to be desired. In the first place, there are almost as many definitions of "laterite" as there have been authors and travelers reporting their observations in the tropics. Some writers seem to have considered that any soil in the tropics, particularly a red one, which appeared unusual to the pedologist trained in the temperate zone, should be grouped with the "laterites"; some have even included most of the vast and uncertain group of "terra rossas" with "laterite." Others have tried to use the difficultly determined and often meaningless criteria of silica/sesquioxide, silica/alumina, or similar ratios to determine whether or not a soil is a "laterite" or is "lateritic." Only very few students of equatorial soils have had the courage to ignore these and other general and largely erroneous conceptions as to the nature of laterite, and to limit their use of the term "laterite" to the more or less indurated, illuvial, quarryable horizon in the soil resulting from the accumulation of ferric oxides (1; 14, pp. 369-390; 15).

By contrast, it is rather the various interpretations of Harrassowitz's hypothesis as to the nature and mode of formation of laterite which continue to be generally accepted by European temperate-zone students of equatorial soils. It should be remembered that, as far as we are aware, Harrassowitz, never having had an opportunity to study at first hand the soils of equatorial regions, had to depend upon others for field observations and collection of samples. At least some of the observations Harrassowitz used were hardly made by those competent to interpret tropical soil profiles.<sup>3</sup> Thus it is easy to understand the divergencies

<sup>1</sup> The original manuscript of this paper was posted in Bangkok, Siam, on November 13, 1941. It never reached the editor of *SOIL SCIENCE*. In April 1946 a copy was located in the Department of Agriculture, Bangkok, and is presented herewith. In the interim the name of the Kingdom has been changed back from Thailand to Siam.

The second paper of the series, by the same authors (17), posted a fortnight later, arrived safely and was published.

<sup>2</sup> The senior author is now soil scientist, Technical Collaboration Branch, Office of Foreign Agricultural Relations, U. S. Department of Agriculture. The junior author has now retired from Siamese Government Service.

<sup>3</sup> In conversation with the senior author in Amsterdam in 1935, Prof. E. C. Jul. Mohr said that in a then recent conversation with Professor Harrassowitz, the latter had admitted that

between Mohr's and Harrassowitz's hypotheses as to the manner of the formation of laterite and its actual character.

The situation has been further clouded and confused by an astonishing lack of consistency between various writers in the definition and use of other terms in their discussions regarding equatorial soils. Naturally, it has been quite impossible for temperate-zone students of the literature to deal critically with the resulting mass of unrelated and often apparently contradictory material at their disposal. Some of the more discerning writers have appreciated that in the absence of more first-hand data their efforts to arrive at and to present a clear idea of the nature of laterite, of laterization, and related phenomena are well-nigh hopeless.

It becomes more and more clearly evident that until there are available to students of soils in the temperate zones adequate first-hand field knowledge and detailed descriptions of the physical character of laterites and laterite soil profiles and chemical analyses of equatorial soils, further attempts to generalize on the characteristics of equatorial soils or to develop theories of the principles of soil formation in equatorial regions will continue to make confusion of the subject worse confounded. This is illustrated all too well in Mattson's (10, p. 415) attempt to account for the *reputed* characteristics and supposed manner of formation of tropical soils, and especially of laterites.

If authors could study without *a priori* misconceptions the data and interpretations by Campbell (2) and Mohr (12), the present-day literature on equatorial soils, and especially on laterite, would emphasize very different conceptions and theories—at least the perpetuation of the most obviously incorrect hypotheses would cease.

In this paper, therefore, emphasis is placed upon the authors' own data and their significance in the light of the better qualified but less well known students of laterite and laterite soils who have studied equatorial soils *in equatorial regions*.

#### OBSERVATIONS AND ANALYSES OF SIAMESE LATERITES

Because of the scarcity of descriptions of laterite soil profiles in the literature, it seems worth while to place on record some of the field observations on the laterites of Siam, together with the chemical analyses of samples collected in the course of field studies. Many of the samples, the analyses of which are reported here, have been figured recently (16). Specific references will be found in table 1.

Many of these samples were collected from ancient buildings or ruins. Others are samples of laterite exposures where, as a result of erosion or for other reasons, the material when collected no longer retained its place in the normal laterite soil profile.

Chemical analyses, profile descriptions, and illustrations of complete laterite soil profiles have been presented in a separate paper (17).

In table 1 are given some notes as to the nature of the laterite samples and the

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in view of the observations and their interpretation presented by Mohr (12) there was very little of Harrassowitz's hypothesis which could stand.

locations where they were collected. The analyses of these laterites are presented in table 2.

#### METHODS OF CHEMICAL ANALYSIS

A rather too vigorous grinding of the samples usually preceded passage through the 2-mm sieve, which is the general method of preparing the sample for the various laboratory tests.

Silica and sesquioxides were determined by methods recommended by the Association of Official Agricultural Chemists. Iron was volumetrically estimated by the reduction of titanous chloride ( $TiCl_3$ ).

Determinations of Ca, Mg, K, and Ti were also made. As the quantities of of these elements found were usually small, and as the quantities and variations found between the various samples do not appear to indicate anything significant, the data have not been included in this paper.  $TiO_2$  scarcely ever exceeded 1 per cent. Unfortunately, Mn, which might have shown certain interesting trends, was determined in only a very few of the samples.

Despite the fact that it has thus far been impossible to determine definitely the sites from which the laterites used in the ancient structures were quarried, and since in most cases it is even difficult to state with any certainty the character of the parent materials from which laterites still in place have been derived, an attempt has been made to subdivide the results of the analyses in table 2. In this table the first group is of samples of laterite building blocks, mostly from very ancient buildings and ruins. The samples have been divided into four subgroups, depending upon the prevailing type of country rock or other parent materials from which the laterite is presumed to have been developed, provided it was quarried in the locality where it was used in construction. In the case of the laterite used in ancient buildings in Ayutia, for example, the material may have been brought down the river by boat very considerable distances. Inasmuch as the second group of analyses in table 2 are of samples of laterite collected in the field, it is possible to state the general type of country rock or parent material, though with the profound weathering which preceded laterization, one can be certain of the actual parent rock only in the small group of laterites derived from "basalt rock."

It is interesting to note how the proportions of  $SiO_2$  in the samples vary with the nature of the country rock. Only 3 of the 53 samples in this table have a higher proportion of  $Al_2O_3$  than of  $Fe_2O_3$ . And the averages of the several subgroups show clearly that in the great majority of cases there are from two to three times as much  $Fe_2O_3$  as of  $Al_2O_3$  present in the samples. Evidently Siam is not a region of bauxitic (high-aluminum) laterites.

Now with some of the chemical characteristics of Siamese laterites before us, let us briefly consider certain additional points regarding laterite in general.

#### GENERAL CONSIDERATIONS REGARDING LATERITES

Soil surveyors fully understand the necessity of having soil profile characteristics which can be identified in the field, hence there is no need here to argue

TABLE 1  
*Descriptions and locations\* of laterite samples analyzed*

86-L	Laterite block from the late 11th century "Buddhist Temple" (cf. <i>Geogr. Rev.</i> 31: 180, fig. 2).	Old Sukotai, Sukotai Province, North-central Siam.
156-X	Laterite from the north wall of the ruins, about 900 years old. Concretionary form, more spherical.	Ban Kam Paeng, Utopompawnpisai Township, Sisaket Province, NE Siam.
157-X	Laterite from the south wall, more distinctly honeycomb structure. About 900 years old (cf. <i>Geogr. Rev.</i> 31: 193, figs. 40, 41).	
173-X	Laterite chunks exposed in stream bank, about 2 m. below the level of the valley floor.	About 18 km. north of Kalasin, Mahasarakham Province, NE Siam.
321-X	Laterite exposed in river bed (cf. <i>Geogr. Rev.</i> 31: 187, figs. 15, 16, 17).	Kuen Nieng river, Ratapum Township, Songkla Province, Southern Siam.
321-Y	Similar to 321-X, about 100 m. farther down stream.	
353-X	Laterite ledge exposed in a tract which floods too deeply for padi planting. Soil 0-15 cm. dark gray clay loam; 15 cm. gravelly loam with iron concretions.	Tachin, 4 km. west of Taptieng, Trang Province, Southern Siam.
380-R	Laterite from walls of the 10th century A.D. Wat Kampang Rang (cf. <i>Geogr. Rev.</i> 31: 181, fig. 5; 194, figs. 46, 47).	Petchaburi, Western Siam.
419-L	Laterite ledge exposed by the sea at low tide.	West coast, Kradat Island, Trat Province, SE Siam.
426-L	Reef of laterite exposed at low tide; mollusks grow inside openings (cf. <i>Geogr. Rev.</i> 31: 187, figs. 20, 21), fig. 3.	Northern reef, Kradat Island, Trat Province, SE Siam.
436-L	Laterite in forest on lower slopes of Khao Yai. Only exposure of laterite seen on this island.	Middle of east coast, Gut Island, Trat Province, SE Siam.
437-L	Laterite, the only exposure observed on this island. In a stony stream bed.	1 km. NW of Salakpet village, Chang Island, Trat Province, SE Siam.
443-L	Laterite, hard chunks, lying loose on the surface, 50 m. south of the river.	‡ km. east of Khao Saming Township office, Trat Province, SE Siam.

\* Many of the place names in this paper are spelled differently from those in earlier papers. Not only have there been numerous and confusing changes in the official Siamese names of towns and districts in the Kingdom, but in many cases the "romanized" form differs greatly from the formal or official transliteration into the Latin alphabet.

TABLE 1—Continued

450-L	Surface chunks of laterite exposed along cart road in forest, on slight elevation. In places the fine sandy loam soil has been eroded, exposing the gravelly loam.	2 km. NW of Wat Ta Chang, Khao Saming Township, Trat Province, SE Siam.
460-L	Fragments of path pavement (cf. <i>Geogr. Rev.</i> 31: 201, fig. 63).	Near Wat Blai Klong, Trat Province, SE Siam.
461-L	Fragments from bottom of recently quarried laterite block.	Kam Kraw Island, Central Township, Trat Province, SE Siam.
485-L	Softer laterite from bottom of recently cut building block; from about 1 m. below surface of laterite horizon.	Hin Dat, Tamai Township, Chantaburi Province, SE Siam.
485-M	Laterite too soft for effective quarrying into building blocks "because located too deep below the surface."	
576-L	Laterite block from a quarry, exact depth not known. Surface soil about $\frac{1}{2}$ m. deep (cf. <i>Geogr. Rev.</i> 31: 192, fig. 34).	Kwang Island, Central Township, Chantaburi Province, SE Siam.
579-L	Laterite chunk dug out in preparing soil for planting pepper. A nearby well shows about 1.5 m. gray to light bluish gray sandy loam underlain by 1.5 m. firm laterite mottled and variegated light brown and light bluish, 2 m. softer laterite below, or to a total depth of 5 m. Well much wider near bottom.	Ban Nong Chawi, Khao Bai Si, Tamai Township, Chantaburi Province, SE Siam.
584-L	Vesicular laterite chunk, originally in the wall or columns of the "grand palace."	San Pet, Wat Prasi, Ayutia town, Central Siam.
584-M	Concretionary laterite, a type not often noted in these ruins.	
586-L	Laterite chunk from an ancient ruin on top of hill.	Praputabat hill, north of Paknampo, Nakorn Sawan Province, Central Siam.
589-L	Loose chunk of laterite, on a hilltop, about 25 m. SW of temple.	Wat Pratan, Lap Lae Township, Utaradit Province, Northern Siam.
590-L	Building block cut from laterite; east end of Pratan hill, $\frac{1}{2}$ km. east of 589-L.	
592-L	Building block from prachedi.	Muang Gao, $\frac{1}{2}$ km. south of Luang rapids, Sisachanalai Township, Sukotai Province, Northern Siam.

TABLE 1—Continued

593-L	Laterite block from ancient prachedi (cf. <i>Geogr. Rev.</i> 31: 198, fig. 58).	Near highway, east of Yom River, Khao Glang, Sisachanalai Township, Sukotai Province, Northern Siam.
597-L	Laterite building block from top of a wall; very porous. Most laterite used in this locality has a much larger quantity of quartz inclusions than this sample, which has more a pisolithic structure (cf. <i>Geogr. Rev.</i> 31: 180, figs. 2, 3).	"Brahmin Temple," Old Sukotai, Sukotai Province, Northern Siam.
604-L	Hard exposed laterite, 0-10 cm. depth, a truncated profile; lower, southern slope of a rocky hill (cf. <i>Geogr. Rev.</i> 31: 183, fig. 8. By error the legend for this figure is printed under figure 6).	Talat Pi, Sisachanalai Township, Suktai Province, Northern Siam.
625-L	Laterite chunks exposed about the spring at the base of the slope (cf. <i>Geogr. Rev.</i> 31: 186, fig. 11).	Wat Din Daeng, Dong Praram, Prachinburi Province, East-central Siam.
627-L	Accretionary deposit among the large sandstone boulders, near the stream.	Khao Nam Tok, Prachin Township and Province, East-central Siam.
630-L	Laterite for road metal, from fresh quarry.	South of air field, Prachinburi, East-central Siam.
634-L 634-M	Laterite exposed on the river bank, some chunks fallen, others in place (cf. <i>Geogr. Rev.</i> 31: 186, fig. 14). <i>Clay</i> from horizon 1 m. thick lying just beneath the laterite and above the vertically bedded weathered rock.	Tatum market, Simahapot, Prachinburi Province, East-central Siam.
648-L	Laterite, a chunk lying free on the surface in the open forest.	22 km. east of Tapan Hin, Chon Daen Township, Pechabun Township, Central Siam.
675-L	Laterite building block from an old prachedi.	Ban Goy Prachedi, Makang Township, Udawn Province, NE Siam.

TABLE 1—Continued

678-L	Laterite building block from plinth of an ancient shrine. Superstructure of buff brick with posts and lintels of red sandstone.	Tat Dum, 4 km. NE of town, Sakon Nakorn Province, NE Siam.
687-L	Part of a laterite temple boundary marker; 12th century A.D.	Muang Sam Sip Township, Ubon Province, NE Siam.
714-L	Laterite exposed at the surface in open forest; soil a gravelly loam.	87 km. north of Chiengmai, Huey Chang Tai, Fang Township, Chiengmai Province, Northern Siam.
738-L	Incipient laterite exposed in bank of Ing River (cf. <i>Geogr. Rev.</i> 31:186, fig. 12).	Ban Pong, Terng Township, Chiengrai Province, Northern Siam.
763-L	Laterite, part of the only chunk of shaped, structurally used laterite seen in Prae or Nan Province.	Wat Cha Hang, 3 km. SE of Nan town, Nan Province, Northern Siam.
805-L	Laterite, surface exposure on knoll in region of light colored, light textured fine sandy loams.	Dong Chang Di, 6 km. SE of Utaradit, Northern Siam.
813-L 814-L	Laterite, concretionary or pisolithic type, from an ancient (probably 12th century A.D.) ruin. Laterite, cellular or vesicular type, from the same ruin (fig. 1).	Near railway hotel, Lopburi Province, Central Siam.
841-L 841-Lc	Laterite building block on the bank of the Menam Chao Phya River, below temple. Vesicular or porous structure (?) Another block, pisolithic structure.	Wat Lat Bunnag, Sanphya Township, Chainat Province, Central Siam.
860-L	Laterite fragments near old ruins of temple of laterite and sandstone (cf. <i>Geogr. Rev.</i> 31:193, fig. 43).	Kasetwisai Township, Roi Et Province, NE Siam.
874-L	Laterite block carved into crenelated wall capping (cf. <i>Geogr. Rev.</i> 31:193, fig. 44).	9 km. north of east from Roi Et, NE Siam.
877-L	Laterite from the old wall.	Gu Praguna temple, 5 km. SE of Sawanapum, Roi Et Province, NE Siam.

TABLE 1—Concluded

891-L	Laterite, pisolithic type, from the central shrine, about 10th century A.D. (cf. <i>Geogr. Rev.</i> 31: 194, fig. 48).	Pimai ruins, Pimai Township, about 50 km. east of Nakorn Rachasima, NE Siam.
892-L	Laterite, vesicular type (cf. <i>Geogr. Rev.</i> 31: 194, fig. 48).	
893-L	Laterite building block from the south gate of the Pimai ruins (cf. <i>Geogr. Rev.</i> 31: 195, figs. 50, 51), fig. 6.	

906-L	Concretionary mass from an atypical body of Leon sand. It should be noted that this concretionary mass was considered atypical, for it had none of the characteristics of the organic-iron-cemented podzol horizon typical of the Leon series.	Near Gainesville, Florida, U. S. A.
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about the desirability of restricting the use of the term "laterite" to the originally described material (1; 14, pp. 369-390).

Though not given the attention they justly deserve, the studies of Campbell (2) and Mohr (12, 13) explain quite satisfactorily how laterite develops in relation to the water table; why the formation of the laterite horizon is limited vertically to a relatively thin zone within the soil; and what the relative positions of the Fe and Al compounds are in the laterite profile.

Despite Campbell's clear explanation of the nature and manner of formation of laterite, the puzzling thing is how such an outstanding soil morphologist as Marbut, after so many years of field experience with temperate-zone soils could have made the mistake of establishing a purely chemical definition of laterite (7, pp. 115-221; 23). Not long after they wrote this definition, Marbut and Manifold made extensive observations on soils in the Amazon Valley (8). There they saw what they called "ground water laterite" and correctly interpreted its manner of formation. Mentioned in lectures in Washington, D. C., as early as 1928<sup>4</sup>, Marbut's conclusions as to the nature and manner of formation of "ground water laterites" and related tropical soils were not formally announced until 1930 and were published in 1932 (9). There appear to be no essential differences between laterite, as Mohr describes it, and Marbut's "ground water laterite," for Marbut observes that the ground water laterites of Brasil have developed in soils normally occurring on a peneplain where, even during long periods of time, there is neither appreciable soil erosion nor material change in the location of the zone of fluctuation of the upper surface of the ground water. Though Marbut had gone a long way toward a complete clarification of the questions of the nature and mode of formation of laterite, for he calls the Harrassowitz profile abnormal, he did not seem to be able to free himself completely from other temperate-zone interpretations and conceptions of laterite<sup>5</sup>, for he continued to call the Cuban

<sup>4</sup> According to James Thorp, in a private communication.

<sup>5</sup> There is not known to be any true laterite horizon in any of the soils in the continental United States. In 1939 Professor Henderson of the Florida Agricultural Experiment Station was very generous with his time and transportation facilities and took the senior author

TABLE 2  
Silica, iron, and alumina in Siamese laterites\*  
Quarried laterites in structures

SAMPLE NO.	SiO <sub>2</sub> per cent	Fe <sub>2</sub> O <sub>3</sub> per cent	Al <sub>2</sub> O <sub>3</sub> per cent	SiO <sub>2</sub> Fe <sub>2</sub> O <sub>3</sub> + Al <sub>2</sub> O <sub>3</sub>
<i>Regions of sandy soils</i>				
156 X	21.7	48.7	17.7	0.75
157 X	37.3	35.2	12.4	1.8
592 L	82.2	10.0	4.8	12.5
593 L	51.9	27.3	12.4	2.9
597 L	64.8	18.4	10.8	4.9
604 L	49.3	1.6	27.8	2.9
860 L	47.4	35.3	9.2	2.5
874 L	32.2	45.6	10.4	1.1
877 L	41.3	35.2	10.7	2.3
891 L	46.6	31.4	18.3	2.1
892 L	48.3	32.0	9.0	2.8
893 L	37.9	40.7	9.0	1.8
Average	47.0	30.1	12.71	3.2
<i>Regions of basaltic country rock</i>				
485 L	29.8	31.5	24.8	1.1
485 M	17.4	48.2	18.7	0.6
Average	23.6	39.9	21.8	0.85
<i>Parent materials of mixed origin</i>				
86 L	40.7	36.7	15.6	1.7
380 R	53.5	29.6	10.3	3.2
160 L	22.2	44.6	19.8	0.78
576 L	23.7	42.8	20.5	0.85
589 L	24.7	38.8	22.2	0.89
590 L	23.0	46.9	18.1	0.82
Average	31.3	40.0	17.7	1.37
<i>Unknown parent materials</i>				
584 L	25.5	41.4	19.9	0.94
584 M	21.0	47.1	16.3	0.77
586 L	30.6	42.0	11.7	1.3
675 L	28.6	49.2	13.0	1.1
678 L	30.3	51.7	14.3	1.1
687 L	33.2	52.0	11.6	1.3
763 L	48.2	34.0	1.5	3.5
813 L	37.6	39.9	12.2	1.7
814 L	68.5	19.2	7.2	6.0
841 L	56.0	29.6	7.6	3.6
841 Le	37.4	37.2	13.0	1.7
Average	37.9	40.0	11.9	2.1

TABLE 2—Continued  
Laterites collected in the field

SAMPLE NO.	SiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	$\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3}$
	per cent	per cent	per cent	
<i>Regions of sandy soils</i>				
173-X	40.9	38.2	11.2	1.9
436-L	52.9	28.3	11.8	3.0
579-L	32.6	38.4	16.4	1.4
648-L	40.2	34.4	14.3	1.9
805-L	34.0	38.9	15.7	1.4
906-L	60.4	27.5	4.6	4.6
Average.....	43.5	34.3	12.3	2.36
<i>Basaltic country rock</i>				
419-L	13.0	44.9	22.6	0.43
426-L	7.1	60.4	14.0	0.23
Average.....	10.1	52.7	18.3	0.33
<i>Parent materials of mixed origin</i>				
321-X	33.1	38.9	17.9	1.3
321-Y	29.3	37.9	22.5	1.1
353-X	20.9	38.5	27.7	0.68
437-L	45.6	34.2	12.5	2.3
443-L	20.0	46.4	19.4	0.69
450-L	28.1	52.1	9.1	1.1
461-L	25.8	43.1	19.3	0.94
625-L	28.4	40.6	19.2	1.1
627-L	47.3	28.7	14.0	2.5
630-L	38.2	39.0	13.3	1.7
634-L	33.0	41.5	15.5	1.3
634-M	74.5	12.0	7.5	8.4
714-L	28.7	10.4	46.4	0.92
738-L	61.7	7.0	22.5	3.9
Average.....	36.8	33.6	19.1	2.0

\* Analysis by carbonate fusion.

Nipe series profile, developed under conditions of free drainage and a very deep water table, a "normal ferruginous laterite."

many hundreds of miles to see typical soils of the Tifton series in the vicinity of Tifton, Georgia. The Tifton series is considered by the U. S. Soil Survey to be the most thoroughly weathered and best example of "laterite" soil in the United States. And though the senior author saw a number of profiles which showed a more or less clear reticulate mottling or flaming, he did not see a single profile which he felt showed more than the commencement of development of a distinct laterite horizon.

Apparently because of the chemical conceptions he had then only recently developed in the course of his study of the data relating to the soils of Africa (7, pp. 115-221), Marbut did not seem to realize that his "ground water laterite" was the true laterite, or that all chemical definitions of laterite should be discarded because (a) such definitions are entirely inadequate; (b) they cannot be applied in the field; and (c) they lead to hopeless confusion. Though it seems a pity, it is understandable that the U. S. Soil Survey continues to hold to Marbut's definition of laterite (23). As long as the world's leading soil survey organization holds to this and certain other conceptions, a full and clear understanding of equatorial and related soils will be retarded.

In the study of equatorial and other soils, entirely too much emphasis has been placed upon the silica/sesquioxide ratios of different parts of the profile, particularly where no attempts or only inadequate ones have been made to separate the different forms of Si. From a field study of the profiles which Harrison (4) analyzed so carefully in the chemical laboratory, Milne (11, pp. 19-20, 60-64) has drawn attention to the heterogeneous character of the parent materials and to the undue emphasis Harrison had placed upon the different percentages of Si he found in these profiles. "Resilication" of horizons in laterite soil profiles is thus believed to be of much less importance than most writers are wont to ascribe to it.\*

The analyses reported in this paper, showing the predominance of  $\text{Fe}_2\text{O}_3$  over  $\text{Al}_2\text{O}_3$  and the relatively small amounts of the latter in most of our laterites show that it is a mistake to define laterite in terms of the  $\text{Al}_2\text{O}_3$  content (5).

#### THE ONLY CORRECT DEFINITION OF LATERITE

In considering the descriptions of the samples and the analytical data, one should clearly understand that by *laterite* is meant the material originally described by Buchanan (1) and later more fully by Oldham (14, pp. 369-390), namely, an illuvial horizon, largely of iron oxides, with a slaglike cellular or pisolithic structure, and of such a degree of hardness that it may be quarried out and used for building construction (figs. 1, 2).

With only occasional exceptions, the *laterite horizon develops within the soil*, in the zone in which the upper surface of the water table fluctuates. Where laterite is exposed at the soil surface (some authors call this an "ironstone pavement") it is, with few exceptions, the result of the soil profile's having been truncated, that is, erosion had carried away the overlying soil—the eluvial horizon.

Pendleton has elsewhere (15) given his reasons for thus restricting the definition of laterite. He has also described and illustrated (16) how laterite occurs in

\* Just to hand is a paper by Dr. F. Whitehouse: Studies in the Late Geological History of Queensland. University of Queensland, Department of Geology 2 (n. s.), No. 1. 1940. In his description of the lateritic soils of Western Queensland (part 1) Whitehouse presents abundant evidence of the importance of the precipitation of silica, there termed "billy," in the development of certain lateritic soils of that region. We must therefore admit that under at least certain circumstances "resilication" of certain horizons in the soil profile is of importance.

Siam and Cambodia, and how it is quarried and has been used in ancient buildings. A *laterite soil* is one in which a laterite horizon is found in the profile. A *lateritic soil* is one in the profile of which there is an incipient or immaturely developed laterite horizon, and in which it is believed a true laterite horizon will develop if the prevailing conditions persist long enough. However, the incipient



FIG. 1. THE 12TH CENTURY A.D. PRA PRANG SAM YAWT TEMPLE, LOPBURI, CENTRAL SIAM  
STILL RETAINS SOME OF THE ELABORATELY DECORATED PLASTER FINISH  
WHICH ORIGINALLY COVERED ALL OF THE LATERITE MASONRY

Only the posts and lintels of the doorways and the finials on the towers are of stone. Some of the laterite masonry had recently been pointed with white mortar (cf. *Geogr. Rev.* 31: 195, fig. 49).

laterite horizon, though slightly firmer than other horizons in the profile, is far too soft and crumbly to be quarried. Such an immature laterite horizon has the typical variegated coloring ("flamed," "mottled," "splotted," etc.) with the red, purplish, or brown iron oxide deposits rather firmer and more resistant to erosion than the white or slightly bluish "clay" which fills the interstices or

tubules (8, 9, 16, 23). Much has been made of the supposed property of laterite to harden on exposure after quarrying, and probably it does become somewhat firmer. The laborer hired by Pendleton (16) to quarry laterite always placed the freshly quarried blocks in the sun for several days, but there appeared to be no marked hardening of the material. Most laterite is usually easily shatterable when struck a sharp blow with a sledge hammer.



FIG. 2. LOOKING UP THE GENTLE SLOPE ACROSS THE LATERITE WHICH HAS HERE BEEN EXPOSED PARTLY BY EROSION AND PARTLY FROM QUARRYING BLOCKS FOR WELL CURBING AND OTHER PURPOSES

The laterite horizon lies just under the grass, or protrudes slightly here and there in the distance. Hin Dat, Tamai Township, Chantaburi Province, Southeastern Siam.

Though laterite usually develops in a peneplain, it is frequently only after a lowering of base level, and after erosion has begun to remove the looser covering eluvial horizon of the soil that the presence of laterite in the soil of a region is really appreciated, for by that time long exposure of the laterite to the sun's heat and to the atmosphere has somewhat hardened it.

Under peneplain conditions, where laterite normally develops, the water

table fluctuations are more or less uniform over a very long time, but in the soil of gentle slopes about the base of hills or slight elevations, where ground water seeps laterally outward and carries ferrous compounds, these may also oxidize and precipitate in the soil to form laterite. In such cases it seems possible that the laterite might actually build upward, through lateral movement of soil moisture carrying ferrous compounds out over a previously developed laterite horizon,



FIG. 3 THE BROKEN FACE OF A PART OF A LATERITE REEF COLLECTED DURING LOW TIDE ON KRADAT ISLAND, SOUTHEASTERN SIAM

Mollusk shells are still in some of the cavities where they grew Sample 426 L

until it reaches the surface of the ground. It is more likely, however, that under these conditions, as soon as the illuvial horizon is sufficiently developed and impervious, the soil above the laterite becomes saturated during very heavy rains, and with percolation impossible, the overlying soil is washed away. The two most conspicuous exposures of laterite thus far noted on the surface in Siam—at Talat Pi, Sisachanalai, Sukotai, and at Hin Dat, Tamai, Chantaburi (19, figs. 6 and 8)—appear to have been formed in this way (fig. 2).

It should be clearly understood that unless a soil has an indurated laterite horizon the authors do not call it "a laterite soil." They do not consider even as lateritic the very friable red, granular lixivium soils, which are said to resemble the



FIG. 4 PART OF A QUARRIED BLOCK OF VESICULAR LATFRITE FROM THE CITY WALL, BUILT ABOUT 150 YEARS AGO, MUANG GAO, CHANTABURI PROVINCE, SOUTHERN SIAM



FIG. 5 PART OF A BLOCK OF LATRITE FROM A WALL AT THE BASE OF THE HILL ON THE EASTERN SLOPE OF Pnom Bakheng, A 9TH CENTURY A.D. BUILDING SOUTH OF ANGKOR THOM, CAMBODIA

This sample contains some included cobblestones, one larger than a hen's egg.

Nipe series of Cuba. Following Prescott (20), they include such clays with the tropical "red loams," for they are distinctly *loamy in structure*. Where equatorial (tropical) "red loams" of this general type have large chunks of cellular, slaglike laterite only at the top of the profile, that is, lying on top of the "red

"loam" as in Surigao, Philippine Islands (3)<sup>7</sup> the explanation is, as Milne points out (11), that this laterite was formed when the ground water was close to the surface of the ground, before the base level of the region was lowered. After the lowering of the base level, that is, after elevation of the land much higher above the sea level, and the erosion of deep ravines so that the ground water is not retained within the oxidizing zone but is readily percolated downward and away into the sea, the subsequent weathering of the deeper materials results in the formation of the "red loams," really a very friable clay, so rich in iron oxides that it is at times in demand as an iron ore.

Since laterite is an illuvial deposit of iron oxides, precipitated within the soil where this is moistened by the more or less oscillating upper surface of the ground water, it is obvious that the physical nature of the soil material in this zone may

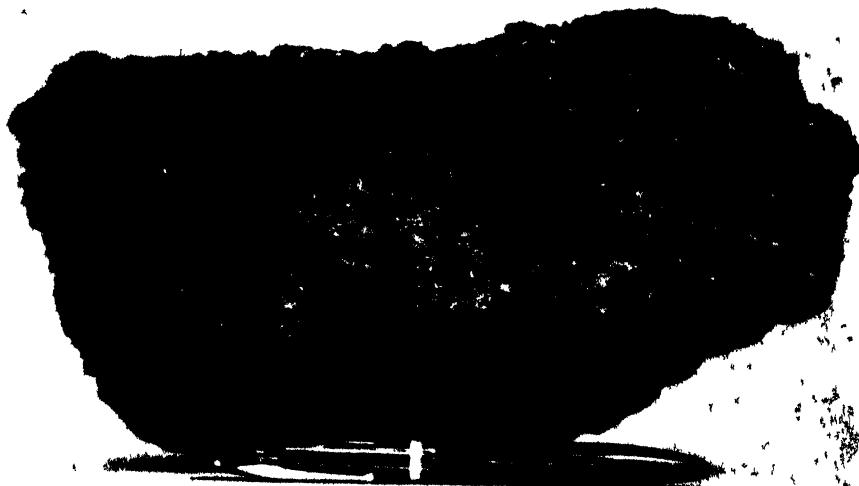


FIG. 6. NODULAR OR PISOLITIC LATERITE WITH LARGER CAVITIES, FROM THE SOUTH GATE OF PIMAI, NAKORN RACHASIMA PROVINCE, NORTHEASTERN SIAM,  
BUILT IN THE 9TH AND 10TH CENTURIES A D SAMPLE 893-L

easily affect the character of the deposit, and that a major portion of a relatively insoluble matrix, such as quartz sand or quartz gravel, will be mechanically included in the laterite.

Though the physical form of laterite varies a great deal in Siam (16, 18), there appear to be two main types, the vesicular (figs. 3, 4, 5) and the pisolithic (fig. 6), with many intermediate sorts. The much more common vesicular type has probably been developed in a clayey or mixed material. The holes left by plant roots or insects serve as the channels by which the air gains access to the ferrous compounds, precipitating the iron as ferric compounds. The pisolithic

<sup>7</sup> Samples of the laterite were shown to the senior author and described by Dr Dean Frasché, geologist of the Philippine Commonwealth, in personal conversation, June 1939. The main earthy or clayey mass of these Surigao ores is said to be very similar to the Nipe soils of Cuba.

type of laterite usually seems to develop in a sandy matrix where the solutions of ferrous compounds can move through the mass more readily, as also can the oxidizing air.

#### SUMMARY AND CONCLUSIONS

Divergencies in the definition of "laterite" have arisen largely because of inadequate data and because of insufficient first-hand knowledge of a representative range of tropical soil profiles.

Extensive travel in many parts of Siam and neighboring countries has given the senior author an opportunity to observe the manner of occurrence of laterite, how it is quarried, and how it has been used in ancient structures.

The term "laterite" is used throughout this paper in the original, restricted sense; namely, it is the indurated slaglike or pisolithic, iron-oxide-rich, illuvial horizon in the soil, of such a physical character that the material can be quarried out and used structurally.

Numerous samples of laterite from ancient ruins and from natural outcrops or other occurrences, which have been collected in the course of these travels, have been analyzed by the junior author for their total content of silicon, iron, and aluminum.

When the analytical results are arranged into groups according to the probable character of the parent material, some interesting trends are evident. In all but three cases, the iron content is much higher than the aluminum, the  $\text{Fe}_2\text{O}_3$  content averaging from two to three times that of the  $\text{Al}_2\text{O}_3$ . Obviously, definitions of laterite which place the emphasis upon its aluminum content are misleading. The validity of the grouping used in table 2 seems to be justified. As might be expected, there is a wider range between the averages of the different groups of samples of laterite which were collected in place than in those of laterites collected from ancient structures, which may have been transported considerable distances and as to the probable character of the parent materials of which nothing is known.

#### REFERENCES

- (1) BUCHANAN, F. 1807 A Journey from Madras Through the Countries of Mysore, Canara, and Malabar. 3 v. London. [Especially 2: 440, According to Oldham (14, p. 369).]
- (2) CAMPBELL, J. M. 1917 Laterite: its origin, structure, and minerals. *The Mining Mag.* (London) 17: 67-77, 120-128, 171-179, 220-229.
- (3) FRASCHÉ, D. F. 1941 Origin of the Surigao iron ores. *Econ. Geol.* 36: 280-305.
- (4) HARRISON, J. B. 1934 The Katamorphism of Igneous Rocks under Humid Tropical Conditions. Imperial Bureau of Soil Science, Harpenden, England.
- (5) Imperial Bureau of Soil Science 1932 Laterite and laterite soils. *Tech. Commun.* 24.
- (6) JOFFE, J. S. 1936 Pedology. Rutgers University Press, New Brunswick, N. J.
- (7) MARBUT, C. F. 1923 The soils of Africa. [*In* Shantz, H. L., and Marbut, C. F. The Vegetation and Soils of Africa. Amer. Geogr. Soc. Res. Ser. 13. National Research Council and the American Geographical Society, New York.]
- (8) MARBUT, C. F., AND MANIFOLD, C. B. 1926 The soils of the Amazon Basin in relation to agricultural possibilities. *Geogr. Rev.* 16: 414-442.

- (9) MARBUT, C. F. 1932 Morphology of laterites. *Proc. and Papers Second Internat. Cong. Soil Sci.* (1930) 5: 72-80.
- (10) MATTSON, S. 1941 The laws of soil colloid behavior: XXIII. The constitution of the pedosphere and soil classification. *Soil Sci.* 51: 407-425.
- (11) MILNE, G. 1938 A Report on a Journey to Parts of the West Indies and the United States for the Study of Soils. East African Agricultural Research Station, Amani, Tanganyika Territory.
- (12) MOHR, E. C. J. 1933-1938 De bodem der tropen in het algemeen, en die van Nederlandsch-Indië in het bijzonder. (In 6 parts.) K. Ver. Kolon. Inst. Amsterdam Meded. 31; Afd. Handelsmus. 12.
- (13) MOHR, E. C. J. 1944 The Soils of Equatorial Regions, with Particular Reference to the Netherlands East Indies. (Translated by Robert L. Pendleton.) J. W. Edwards, Ann Arbor, Mich.
- (14) OLDHAM, R. D. 1903 A Manual of the Geology of India. Second edition revised. Government of India, Calcutta.
- (15) PENDLETON, R. L. 1936 On the use of the term laterite. *Amer. Soil Survey Assoc. Bul.* 17: 102-108.
- (16) PENDLETON, R. L. 1941 Laterite and its structural uses in Thailand and Cambodia. *Geogr. Rev.* 31: 177-202.
- (17) PENDLETON, R. L., AND SHARASUVANA, S. 1942 Analyses and profile notes of some laterite soils with iron concretions of Thailand. *Soil Sci.* 54: 1-26.
- (18) PENDLETON, R. L. 1942 Laterite or Sila Laeng, a peculiar soil formation. *Thai Sci. Bul. (Bangkok)* 3: 61-77.
- (19) POLYNOV, B. B. 1937 The Cycle of Weathering. (Translated from the Russian by Alexander Muir.) London.
- (20) PRESCOTT, J. A. 1931 The soils of Australia in relation to vegetation and climate. *Council Sci. and Indus. Res. [Melbourne] Bul.* 52: 45-51.
- (21) ROBINSON, G. W. 1932 Soils: Their Origin, Constitution and Classification. Thomas Murby & Co., London.
- (22) de' Sigmund, A. A. J. 1938 The Principles of Soil Science. (Translated by Arthur B. Yolland.) London.
- (23) THORP, J., AND BALDWIN, M. 1940 Laterite in relation to soils of the tropics. *Ann. Assoc. Amer. Geogr.* 30: 163-194.
- (24) U. S. Department of Agriculture 1938 Soils and Men: Yearbook of Agriculture. U. S. Government Printing Office, Washington, D. C.
- (25) VAGELER, P. 1933 An Introduction to Tropical Soils. (Translated by H. Greene.) London.

# A NEW TECHNIQUE FOR STUDYING THE ABSORPTION OF MOISTURE AND NUTRIENTS FROM SOIL BY PLANT ROOTS

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The ability of plant roots to absorb water and nutrients from subsurface soil depths and to absorb nutrients present in dry topsoil came into question in connection with extensive irrigation and fertility experiments on guayule (*Parthenium argentatum* A. Gray). Muller (4) found that the roots of guayule penetrated the soil to depths as great as 15 feet during the second year of growth in the field and that older plants occasionally reached depths of more than 20 feet. Weaver (7) reported the attainment of similar depths by alfalfa. The behavior of plant root systems in the absorption of moisture and nutrients from the lower depths of the soil profile is of fundamental scientific interest and also of considerable practical importance, since in irrigation and fertility experiments close control can be had over the soil to a depth of only a foot or two below the surface.

There have been few carefully controlled experiments to determine the depths from which plants absorb water and nutrients from soil. Weaver, Jean, and Crist (6) in 1922 grew certain crop plants in large vessels containing soil divided into layers separated by paraffin-petrolatum seals. The depths from which moisture and nutrients were removed were determined by initial and final analysis of the several soil layers.

The recent development of the Richards tensiometer (5) and the Bouyoucos block (1), by which soil moisture can be continuously measured *in situ*, and the advent of artificially radioactive elements in quantity, provide tools for the study of plant root behavior by techniques not previously possible. This paper reports a technique that was developed to make use of these tools in the study of the absorption of moisture and nutrients by plant roots. The extent of the problems that can be studied by the new technique is indicated by the following questions which it was designed to investigate:

1. Starting with soil moisture at low tension throughout the whole profile penetrated by plant roots, is water removed from the topsoil down to the wilting point range before appreciable absorption occurs at lower depths, or is it absorbed at more or less uniform tension throughout the depth of root penetration?

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2. If water is being absorbed at medium tension from a particular level in the soil profile, while the moisture at a second level is held at a considerably higher tension, will the plant continue to absorb water from the first location after the soil at the second location has been irrigated to a low moisture tension?

3. If fertilizer elements are applied to the topsoil and if the topsoil moisture is in the wilting point range but that of the lower horizons is held at low tensions, can the plant absorb water from the subsoil and use that water to effect the absorption of nutrients from the dry topsoil?

4. If the moisture in the topsoil is held at tensions in the wilting point range, while that of the lower horizons is held at lower tensions, does absorption of nutrients from the lower horizons occur?

5. If the moisture is held in the upper soil horizons at tensions in the wilting point range, while that in the lower horizons is at medium or low tensions, will the plant absorb sufficient moisture from the lower depths to maintain growth and vigor?

6. Are nutrient elements absorbed from any part of the soil profile without the absorption of water?

7. If moisture throughout the soil profile is held at low tensions, does the plant absorb nutrients from the lower levels?

8. Can roots remain alive for prolonged periods of time in soil at or below the wilting point? If so, are they able to absorb water and nutrients immediately upon addition of the same?

Curtailment of the research activities of the Special Guayule Research Project and unavoidable circumstances have prevented the authors from continuing their studies to the point of conclusive answers to all the foregoing questions. This report is given to describe the technique that was developed and to present the preliminary findings that were obtained.

#### DESCRIPTION OF TECHNIQUE

Apparatus was designed for the growing of plants in a long column of soil divided horizontally into sections, with independent means of moisture measurement and independent control of moisture and nutrient conditions in each section. The essential features of the experimental setup are shown in the photographs of figure 1.

Three double-walled redwood boxes, 6 by 6 by 72 inches in inside dimension, were prepared as shown. One side of each box was made up of nine 8-inch removable sections attached by screws. The boxes were filled with a mixture<sup>2</sup> of equal parts of Chualar loam, sand, and fine gravel. The soil columns were divided into nine 6- by 6- by 8-inch sections (each containing 7.5, or 8, kgm. of the soil-sand-gravel mixture, hereinafter called "soil") by means of membranes 3 to 5 mm. thick made by brushing a mixture of equal parts of tar<sup>3</sup> (road oil) and paraffin (Parowax) on coarse cheesecloth. These membranes were permeable to plant roots but restricted the movement of nutrients and moisture between soil sections. A perforated plastic tube (with closed distal end) for the introduction of nutrients and moisture and for aeration, and a tensiometer and a Bouyoucos

<sup>2</sup> This mixture was used in an effort to obtain a "soil" having such a pore-size distribution that it would hold appreciable quantities of water throughout the range of moisture tension up to 15 atmospheres.

<sup>3</sup> The use of tar as a component of this mixture was suggested by A. C. Hildreth.

block for the continuous measurement of soil moisture *in situ*, were installed in each soil section. For convenience in care and observation, the boxes were set into a pit 2 feet deep.

The tensiometers were adapted from the original design of Richards (5). The mercury cups, copper and glass capillary tubing, and graduated scales are shown in the photograph at the left in figure 1. Further details of the tensiom-

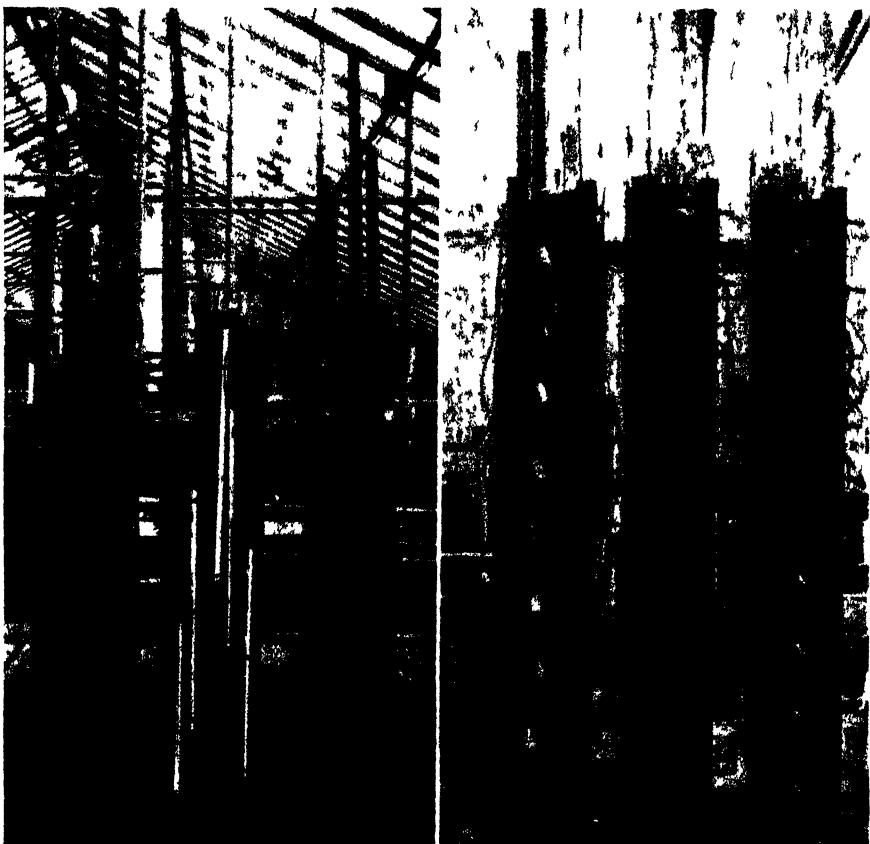


FIG. 1 EXPERIMENTAL APPARATUS FOR ROOT STUDY

Left, 72 inch boxes installed in greenhouse pit Right, boxes and plant root systems after detachment of side of box and removal of part of soil by washing The arrangement of membranes, tensionmeters, Bouyoucos blocks, and plastic tubes is apparent

eters and their installation may be seen in the photograph at the right in the same figure. (In the box at the extreme right six large tensiometer cups, K-948, were employed, the remainder were of the smaller type, K-480.) For each section the setting of the zero point on the scale was obtained with the tensiometer cup in place and partly immersed in water before the addition of soil to that section. The tensiometer cups and Bouyoucos blocks were located near the centers of the soil sections. The plastic tubes were wrapped with glass wool to

prevent the entrance of soil and were given a slight slope to facilitate the flow of water. Glass fittings were attached to their outer ends for ease in making water additions.

The soil in each section was moistened to approximately the "field capacity" and was packed firmly, as the installation proceeded upward from the bottom of the boxes, to prevent later settling which might break the seal between the tar membranes and the box walls to which they were cleated. In all cases the soil on both sides of the membrane remained in contact with the membrane throughout the experiment. In May, 1944, alfalfa was seeded in one of the boxes, and three guayule seedlings were transplanted into each of the two others.

Frequent determinations of the moisture status in the several soil sections were made by means of the tensiometers and Bouyoucos blocks, and, until the roots had penetrated deeply, water was added as required to maintain the soil moisture at low tensions, favorable to root growth. After the roots had reached the lower sections, studies were made involving differential moisture tensions in parts of the soil column, and some investigations of nutrient absorption were made by the use of radiophosphorus and radiostrontium.<sup>4</sup>

#### RESULTS AND DISCUSSION

The use of the tensiometer and the Bouyoucos block makes it possible to determine soil moisture conditions continuously with considerable accuracy throughout the range of moisture available to plants. The tensiometer is sensitive to changes in moisture tension from 0 to approximately 0.85 atmosphere (5). The maximum sensitivity of the Bouyoucos block is reached near the permanent wilting percentage or 15-atmosphere range. In that range its electrical resistance is of the order of 100,000 ohms or more. Since it is composed of gypsum, the Bouyoucos block may not be used in studies involving calcium or sulfur nutrition.

The downward development of the root systems of the plants was evidenced by the reactions of the moisture-measuring instruments. The tensiometers in the several sections registered continuously low tensions, almost constant, until some time after the roots reached the particular soil section in which they were installed. Thereafter the tensiometer readings fluctuated as moisture was withdrawn from the soil by the roots and was replenished by irrigation. Significant variations in the resistances of the Bouyoucos blocks occurred only when absorption by the root systems lowered the soil moisture content to tensions greater than 1 atmosphere.

The approximate time intervals, in days, between introduction of seed (alfalfa) or plants (guayule) into the first or topmost section and tensiometer reaction to soil moisture removal from the lower sections of the soil column are given in table 1. All these are maximum values; undoubtedly some interval of time elapsed after the first penetration of roots into a section of soil before sufficient absorption of moisture occurred to influence the reading of the tensiometer.<sup>5</sup>

<sup>4</sup> The radioactive elements used in this study were supplied through the courtesy of Joseph G. Hamilton, Röntgen Radiation Laboratory, University of California, Berkeley.

<sup>5</sup> In a supplementary experiment to study the speed with which corn roots would penetrate a soil column divided by tar membranes, in 30 days corn plants rooted profusely in all

Several tests were made to study the efficacy of the tar membranes in preventing the movement of water (and nutrients) between adjoining sections of soil. After the top eight sections (64 inches) of soil in one of the boxes growing guayule had been at the wilting percentage for about 10 days, sections 1, 3, and 5 were irrigated to low tensions. For a month thereafter the soil of these sections was irrigated as required to keep the tension below 150 cm. of water (approximately 0.15 atmosphere). Sections 2, 4, and 6 were not irrigated. During the first week of the test, the resistances of the Bouyoucos blocks in sections 2, 4, and 6 did not fall below 250,000 ohms (which was the upper limit of capacity of the conductivity bridge in use at the time). After a month the resistance of the Bouyoucos block in section 2 had fallen to 125,000 ohms, but at no time did that of the blocks in sections 4 and 6 become less than 200,000 ohms. In an analogous test on the other soil box supporting guayule, similar results were obtained. Tensions of the order of 150 cm. of water were maintained for a month in sections 1, 3, 5, and 6. Throughout that period the electrical resistances of the Bouyoucos blocks in sections 2 and 4 remained above 250,000 ohms. These data indicate that the movement of water, upward or downward, across the tar membrane separating soil at very low moisture tension from soil at high tension was very

TABLE 1

*Approximate time interval, in days, between planting of alfalfa and guayule in first section and occurrence of significant fluctuations in tensiometers in lower sections of soil column*

PLANT	TIME INTERVAL BETWEEN PLANTING AND FLUCTUATIONS							
	Section 2	Section 3	Section 4	Section 5	Section 6	Section 7	Section 8	Section 9
	days	days	days	days	days	days	days	days
Alfalfa.....	60	70	105	125	140	150	200	245
Guayule.....	20	35	65	90	125	150	175	300

slight, and in most cases did not occur. The tar membranes were soft and pliable at the temperatures of the greenhouse. Since the roots were actively growing and expanding, they tended to maintain extremely close contact with the perimeters of the holes made in the membranes at the points of penetration. The transport of water through the roots themselves probably explains the slight increase in moisture content observed. In another experiment the authors (3) showed that corn roots were able to penetrate dry soil and to build up its moisture content. Brezeale (2) asserted that plant roots can transport water, give it up to dry soil, and build the moisture content of the dry soil up to the wilting percentage but not higher. Bouyoucos and Mick (1) reported that resistance values of about 100,000 ohms correspond to soil moisture content in the wilting percentage range.

In several instances it was confirmed that there was no appreciable movement

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sections of a similar soil box 40 inches tall which was divided into six sections. Figure 2 shows the relative root development made in 15 months by guayule and in 30 days by corn. The root system of corn develops much more rapidly than that of either alfalfa or guayule.

of water through the membrane from soil at very low tension to soil at the permanent wilting percentage in the section immediately above it. For example,

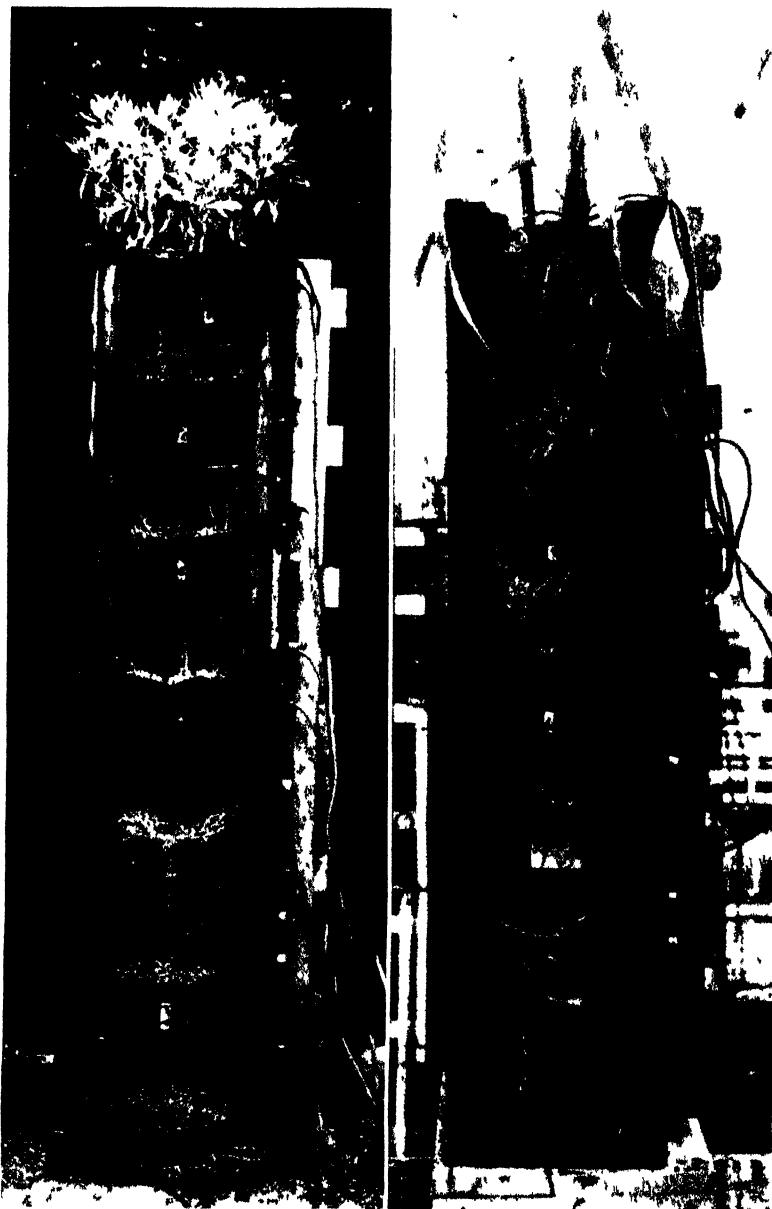


FIG. 2 COMPARATIVE ROOT DEVELOPMENT OF GOMPHRENA AND CORN AFTER  
15 MONTHS AND 30 DAYS, RESPECTIVELY

for 3 weeks the resistances of the Bouyoucos blocks in sections 1, 2, 3, and 4 in the box supporting alfalfa were above 250,000 ohms, while the tension in section

5 and lower sections was kept for the most part below 150 cm. and never rose higher than 312 cm. Observations similar to these were made on the two boxes in which guayule was grown.

Studies of the moisture tension changes in the several sections, as absorption by the plant roots dried the soil column from an initially low tension, were made with both boxes in which guayule was grown. For a period of several weeks (3 weeks in the case of box 2, 8 weeks for box 3) the moisture tension throughout the soil column was maintained at relatively low values (usually below 350 cm. of water) in order that the roots in all sections might be capable of normal absorption. Following this, all sections were irrigated to tensions of about 100

TABLE 2

*Changes in readings of moisture-measuring instruments as moisture absorption by roots dried the soil column from an initially low moisture tension*

DATE AFTER IRRIGATION	INSTRUMENT	UNITS	SECTION								
			1	2	3	4	5	6	7	8	9
<i>Guayule, box 8</i>											
0	Tensiometer	cm. of water	99	114	92	82	104	100	71	81	60
			266	301	172	124	180	129	86	90	64
			548	662	344	216	178	190	104	104	70
			760	780	582	484	260	310	126	119	72
			*	*	632	740	432	440	158	148	86
			*	*	702	*	590	584	232	172	90
11	B. block	ohms	100,000	210,000	31,500	135,000	162,500	30,000	1000	1000	†
<i>Guayule, box 3</i>											
0	Tensiometer	cm. of water	96	118	106	81	97	78	63	82	87
			366	245	230	108	124	81	69	86	86
			752	530	601	180	192	86	76	93	42
			*	720	756	433	400	86	80	94	42
			*	778	*	636	676	92	86	102	46
			*	*	*	810	784	92	90	104	46
10	B. block	ohms	55,000	85,000	82,500	43,000	42,000	600	550	600	600

\* Tension higher than tensiometer range.

† Bouyoucos block not functional.

cm. of water. Changes in moisture conditions in the several sections of the soil column were then measured by the instruments at frequent intervals (hourly, at first) as the plants lowered the moisture content to the wilting point range. After the limit of the tensiometers was reached, further measurements were made with the Bouyoucos blocks. Table 2 presents data obtained in tests on the two boxes supporting guayule.

In general, at a given time the tensions ranged from higher to successively lower values from top to bottom of the soil column. These data indicate that the roots extracted moisture held in the topsoil at fairly high tensions, while moisture was available at lower tensions in the subsoil. The same general be-

havior was noted with alfalfa. The greatest concentrations of roots were present in the upper part of the soil column, as is true under normal growing conditions. It is probable that this was an important factor.

The following experiment was made to study the growth of guayule plants having water available to their roots in the subsurface layers of soil but with the surface soil at the permanent wilting percentage. After a period during which the whole soil column was kept at tensions of less than 1 atmosphere, no further irrigations were made, and during a period of about 3 weeks, moisture was removed by the plants from the six upper sections to such an extent that the Bouyoucos block resistances were in the range of 400,000 to 800,000 ohms. The plants were then defoliated, and during the next 6 weeks water was applied to only the five lower sections. With the top 32 inches of their root systems in soil having a moisture content of less than the permanent wilting percentage, the guayule plants put forth new leaves and continued to grow. Alfalfa behaved similarly, putting forth new shoots while the top 32 inches of soil was at or below the wilting percentage. Growth, however, was considerably less luxuriant than when moisture was available to the plants throughout the soil columns.

Radiophosphorus was used to study the uptake of nutrients by guayule from moist lower sections when the upper part of the soil column was at the permanent wilting percentage or below. After several weeks in which all sections of the column had been kept at low moisture tension, the whole column was allowed to dry to the permanent wilting percentage as indicated by Bouyoucos block resistances of more than 250,000 ohms. Approximately 20 microcuries of radiophosphorus was then added to section 7, 48 to 56 inches from the top of the column. Following this the three lower sections were irrigated and maintained at low tension for several days. Qualitative tests for the presence of radiophosphorus in the aerial portion of the guayule plant were made from time to time, using the Geiger counter. A small amount of radioactivity could be detected on the second day, and relatively large amounts<sup>4</sup> were found in the leaves and stems after a week. This demonstrated conclusively that the guayule plant is able to absorb phosphate from moist soil 48 inches below the surface and transport it to the plant top through 48 inches of soil at or below the permanent wilting percentage.

An attempt was made with the two installations in which guayule was grown to determine whether, if the whole soil column is kept at low tension continuously, the plant will absorb nutrients from the lower levels. Radiostrontium was introduced into the lowest section. During the next 3 weeks, the whole column was kept at moisture tensions of less than 300 cm. of water. No radioactivity due to radiostrontium could be detected in the leaves of the plants. There was very scanty root growth in the lowest sections. The authors feel that for this reason the results of this experiment must be regarded as inconclusive. Under proper conditions the technique that was used is capable of giving a conclusive

<sup>4</sup> Around 800 counts per minute, above the background, when the Geiger tube was held against the leaves of the plants.

answer to the question of nutrient absorption from the lower levels when the soil moisture is at low tension throughout the root zone.

In order to study the absorption of nutrients from topsoil in the wilting point range by plants having ample moisture available to them in the lower part of the soil profile, the following experiment was carried out. The whole soil column of the installation in which alfalfa was growing, and of one of those supporting guayule, were allowed to dry to the permanent wilting percentage, or below, to a depth of 64 inches. A volume of 60 ml. of solution containing approximately 22 microcuries of radiophosphorus and monopotassium phosphate was added at the rate of 400 pounds per 2 million pounds of soil to the topmost section. The water that was applied was soon absorbed, together with a part of the radiophosphorus. After 6 days the alfalfa tops and the leaves and tips of branches of the guayule were removed. Water was then applied to the soil of the six lower sections, that of the three upper sections remaining at the wilting percentage. New shoots were produced. Portions of these were harvested from time to time, and an attempt was made to determine whether the concentration of radiophosphorus in the tops remained constant (disintegration being taken into account), increased, or decreased with time. An increase would have signified that absorption from dry soil was occurring. The data obtained indicate an increase in the radiophosphorus content of the alfalfa tops during the month following the initial harvest made 6 days after the radiophosphorus was added. It is possible, however, that the increase was due to the translocation to the tops of radiophosphorus present in the roots when the initial tops were harvested 6 days after the application of the tracer element. It is felt that the experimental results were not conclusive. An endeavor was later made by a more promising procedure (3) to study the absorption of nutrients from dry soil.

#### SUMMARY

A new technique was developed for the study of the behavior of plant root systems in the absorption of moisture and nutrients from soil. Guayule and alfalfa plants were grown in columns of soil 72 inches tall, divided into 8-inch sections by means of tar-paraffin membranes which were permeable to roots but which restricted the movement of water and nutrients between adjoining sections. Each 8-inch section of the soil column was provided independently with a tensiometer and a Bouyoucos block, for the continuous measurement of soil moisture *in situ*, and a perforated plastic tube for the addition of water and nutrients and for aeration. Water and nutrient elements could be added at will to any section of the columns.

Tests were made to study the efficacy of the tar-paraffin membranes in preventing the movement of water across the membranes from soil at low to soil at high moisture tension. Very little if any such movement occurred. With radiophosphorus it was conclusively demonstrated that the guayule plant could absorb nutrients from moist soil 48 inches below the surface and transport them to the plant top through 48 inches of soil at or below the permanent wilting percentage. There was, in general, a gradient in moisture tension from top to bottom of the

soil column as, beginning with initially low tensions throughout, the absorption of water by the roots lowered the moisture content of the column to the wilting percentage.

#### REFERENCES

- (1) BOUROUCOS, G. J., AND MICK, A. H. 1940 An electrical resistance method for the continuous measurement of soil moisture under field conditions. *Mich. Agr. Exp. Sta. Tech. Bul.* 172.
- (2) BREZZALE, J. F. 1930 Maintenance of moisture equilibrium and nutrition of plants at and below the wilting percentage. *Ariz. Agr. Exp. Sta. Tech. Bul.* 29: 137-177.
- (3) HUNTER, A. S., AND KELLEY, O. J. The extension of plant roots into dry soil. *Plant Physiol.* (In press.)
- (4) MULLER, C. H. Root development and ecological relations in guayule. U. S. Dept. Agr. Misc. Pub. (In press.)
- (5) RICHARDS, L. A. 1942 Soil moisture tensiometer materials and construction. *Soil Sci.* 53: 241-248.
- (6) WEAVER, J. E., JEAN, F. C., AND CRIST, J. W. 1922 Development and activities of roots of crop plants. Carnegie Inst. Wash. Pub. 318.
- (7) WEAVER, J. E. 1928 Root Development of Field Crops. McGraw-Hill, New York.

# SOIL DENSITY AS A FACTOR IN DETERMINING THE PERMANENT WILTING PERCENTAGE

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During some experimentation on irrigation of grapes, a soil without a well-developed profile that seemed to have peculiar water-holding characteristics in the fourth and fifth foot depths was found. Laboratory determinations of moistures equivalents and permanent wilting percentages did not agree with the field capacities and minimum soil-moisture contents found under field conditions. The causes for some of these discrepancies have been pointed out (3).

During 3 years the soil-moisture content at the beginning of the season, about the first of May when the grapes were leafing out, averaged 16.6 and 16.9 per cent in the fourth and fifth foot depths, respectively. Similar samples at the end of the growing season averaged from 13.6 to about 14.4 per cent moisture, showing an average loss of between 2.5 and 3 per cent during about 5 months of the growing season in the unirrigated area. In the irrigated plot, under nearly continuous irrigation, the highest recorded soil moisture was 17.8 per cent, which was but slightly above the average at the beginning of the season. The moisture contents at the beginning of the growing season did not agree with the moisture equivalents, which averaged about 21 per cent. In the unirrigated plot, the loss of moisture from the 4- and 5-foot depths proceeded at a slow rate until between the first and the middle of August, after which it virtually ceased. Cessation of water loss occurred at a point several per cent above the permanent wilting percentage obtained with sunflowers grown on soil removed from these depths with a soil auger. The soil-moisture records for the fourth and fifth feet for a typical season are shown in figure 1.

In later work with native vegetation growing on a primary chaparral soil, a similar situation was found (fig. 2). The soil-moisture content of the third foot was about 12 per cent at the beginning of the season, and was reduced to 10 per cent about the middle of June, where it remained during the rest of the season. The moisture content of the fourth foot remained almost stationary throughout the season, except for slight changes probably due to the variable amounts of clay and gravel in the samples.

The soil in the vineyard is classed by the Bureau of Chemistry and Soils as Bale gravelly loam (2). It is a secondary soil derived from rhyolitic rocks, and contains a moderate to large amount of gravel. The upper subsoil layer to a depth of 36 inches is dull brownish gray and is moderately compact. The lower subsoil is light brownish gray, "slightly" compact, and without structural form or appreciable colloidal staining. Samples taken with a soil tube indicated that the soil at these depths was more compact than described in the soil survey of the area, although it would not be classed as hardpan. Density increased gradually with depth from about 1.38 in the surface soil to about 1.83 in the fifth foot. It is

reported to be a productive soil. The average apparent specific gravity obtained in the field by means of a calibrated soil tube was 1.74 in the fourth foot and 1.83 in the fifth with individual samples as high as 1.88. The relative density and low porosity account for the fact that at no time was the moisture content of this soil more than about 17 or 18 per cent, which is nearly saturation, even though the moisture equivalent of the sample was about 21 per cent. Furthermore, the permanent wilting percentages of the disturbed samples were lower than the minimum moisture content obtained by sampling in the field.

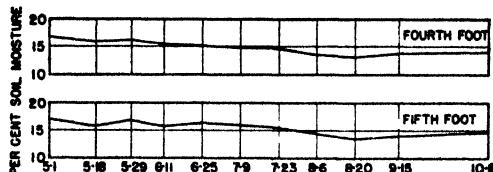


FIG. 1. SOIL-MOISTURE CONTENTS OF BALE GRAVELLY LOAM IN A VINEYARD



FIG. 2. MOISTURE CONTENTS OF A PRIMARY CHAPARRAL SOIL IN AN AREA OF NATIVE VEGETATION

TABLE 1  
*Mechanical analyses of Bale, Holland, and primary chaparral soils\**

SOIL	DEPTH feet	CLAY (<0.005 MM.)	SILT (0.05-0.005 MM.)	SAND (1.0-0.05 MM.)
		per cent	per cent	per cent
Bale	4-5	37.7	22.2	40.1
Holland	2-3	27.2	22.0	50.8
	3-4	26.4	22.0	51.6
Primary chaparral	2-3	31.4	18.6	50.0
	3-4	28.8	18.4	52.8

\* By the hydrometer method of Bouyoucos (1).

The primary chaparral soil also showed increasing density with depth, being about 1.3 in the top foot and 1.9 in the fourth. The moisture equivalent was 20.9 per cent in the third foot and 21.5 per cent in the fourth. Both these values were several per cent above the observed field capacities. On the other hand, on a primary granitic soil of the Holland series having a specific gravity exceeding 1.8 at lower depths, the decrease in soil-moisture content during the season appeared to be about normal.

The mechanical analyses of the Bale, Holland, and primary chaparral soils are given in table 1.

The question arose as to whether these peculiar moisture characteristics were due to failure of the roots to penetrate the Bale and the primary chaparral soils, and also whether other soils might be compacted enough by mechanical means to prevent the entrance of roots.

#### EXPERIMENTAL PROCEDURE

Weighed quantities of Bale, Yolo, and Madera soils were each moistened with a spray of water from an atomizer, the sample being stirred while the spray was being applied. The sample was then tamped into a No. 2 friction-top can until compacted to a predetermined volume. The amount of water used to provide the moisture content that would allow compression to the desired volume varied somewhat with each sample, according to the judgment of the operator. The soil was tamped into the can layer by layer until each layer glistened slightly, indicating the presence of a small quantity of free water on the surface. No claim is made for uniformity of compaction in the whole mass, and the different layers probably varied slightly in density. The data given are the averages for entire samples. Approximately 500 gm. of soil was used for each sample. When the tamping was completed, the surface was covered with a thin layer of wax, consisting of 80 per cent paraffin and 20 per cent petrolatum. Previous experience had shown that sunflower roots would penetrate the wax layer readily. From 120 to 220 gm. of loose soil was placed above the wax seal to provide a favorable medium for starting the plants. This upper layer of soil was then watered and sunflower seeds were planted in it.

The sunflower plants were grown until they were 4 to 6 inches tall and had six to eight leaves, when they were allowed to wilt. The cans were then cut in half longitudinally with a hack saw, and samples taken for moisture and specific gravity determinations. For the latter, blocks of soil were cut out and quickly smoothed and some were dried at 105° C. for 24 hours. These oven-dried and moist blocks were then covered with a thin coat of melted paraffin and the specific gravity was determined in the usual manner. Drying of the compacted soils apparently did not cause appreciable shrinkage, as no cracks in the samples were observed. Furthermore, close agreement in specific gravities was obtained when either moist or oven-dry samples were used.

The three soils used were Bale gravelly loam, previously described; Yolo clay loam, a recent alluvial soil derived from a wide variety of rocks; and Madera loam, a maturely weathered secondary soil of granitic origin with a hardpan at varying depths below the surface. The Yolo and the Madera soils in the field did not show the peculiarities exhibited by the Bale or the primary chaparral soils.

#### RESULTS AND DISCUSSION

The average results from four to six trials for each soil are given in table 2.

When the cans were packed with the moistened soil, an effort was made to compact them to a specific gravity of 1.8. The average results obtained after growth of the plants are given in the last column, which show that one soil was below this value and the other two were above.

By weighing the cans containing the packed soil as soon as the desired compactness was obtained, the moisture content, as shown in column 2, was calculated.

The average moisture contents of the packed soil after growth of the sunflower plants are given in column 3. Fairly close agreement was obtained with the previous values (column 2) for the Bale and the Yolo soils. No roots were found in the compacted layer in the Bale soil, and only a few were observed in the Yolo. Absence of roots was also evidenced by the fact that the moisture content was not appreciably reduced. In the Madera soil, however, the compacted layer was filled with roots, and the moisture content was decreased materially.

Examination of the lower side of the wax seals showed that the roots had no difficulty growing through them. The roots failed to penetrate the Bale soil, however, and penetrated the Yolo only to a slight depth in a few cases. This would suggest that so far as compactness is concerned, there is a limit in these soils, in the vicinity of the value expressed as specific gravity 1.8, beyond which roots do not penetrate. The Madera soil, compacted to a specific gravity of 1.75, allowed the roots to enter and extract moisture; the Yolo soil when compacted to 1.81 allowed a few roots to enter a short distance; and the Bale soil, at a specific

TABLE 2  
*Average moisture contents and apparent specific gravities of compacted soils*

SOIL	MOISTURE WHEN PACKED IN CANS	MOISTURE IN COMPACTED LAYER AFTER PLANTS WILTED	CALCULATED SPECIFIC GRAVITY WHEN PACKED	SPECIFIC GRAVITY AFTER GROWTH OF PLANTS
	per cent	per cent		
Bale (3 to 4 feet)	17.03	16.87	1.89	1.92
Yolo (surface)	19.92	19.19	1.70	1.81
Madera (surface)	20.40	11.58	1.68	1.75

gravity of 1.92, did not permit the entrance of roots. This value of 1.8 does not hold for all soils, as evidenced by the results obtained with the Holland soil.

The absence of roots from the compact lower depths of Bale gravelly loam and from the primary chaparral soil may help to account for the peculiar water characteristic during the three summers under observation. During the early spring months, the soils were nearly saturated, and later slowly drained to field capacity. The small amount of pore space in these soils may explain why they contain relatively small amounts of water in early spring. In view of the clay content and low porosity, the individual pores are probably small. This may explain the slow rate of drainage and the inability of the roots to penetrate the soil. The fluctuation between the amount of water found in the spring and the amount found in the fall may be due to natural drainage without any extraction by roots of plants. If this is the case, a soil-moisture condition comparable to field capacity in ordinary soils may not be reached in certain compact soils until several months after the rains have ceased, instead of within 2 or 3 days, as in well-drained soils.

Moisture equivalents and permanent wilting percentages were determined from

the depths sampled, but do not agree with the results observed in the field. This lack of agreement may be due to the manner in which the samples for laboratory determinations are obtained. The usual method is to obtain a supply of soil by means of a soil auger. Taken in this way, the sample is fragmented to a certain degree. When moistened, either for the moisture equivalent determination or for growth of the sunflower plants, the soil apparently does not again become as compact as it was in the field. In fact, sunflower roots thoroughly permeated the loose soil. For compact soils having an apparent specific gravity of 1.8 or above and small pores, the moisture equivalent and permanent wilting percentages are not determinable by the standard procedure for the former value and by the use of indicator plants for the latter.

It would seem from these experiments that the moisture equivalent and the permanent wilting percentage, in addition to being influenced by texture, are also influenced by the size of the individual pores when the soil is unduly compacted. The apparent specific gravity of 1.8 does not necessarily give any indication of the size of the individual pore spaces. It is probable that other soils, like the Holland, having the same density, might have pore spaces large enough to allow the extraction of water by roots.

It has been pointed out (5) that, in general, there is agreement between moisture equivalent and field capacity of clay soils, but such agreement would not be expected in a compact soil like the Bale because the amount of porosity in the fragmented sample used in the centrifuge is much greater than that which occurs in the undisturbed sample in the field. For example, the Bale soil had a moisture equivalent of about 21 per cent, but was nearly saturated in the field at about 17 per cent, and finally drained to 14 per cent, which may be taken as the field capacity. Work and Lewis (6) also reported higher moisture equivalent than field capacity in heavy soil, the field capacity being nearly at saturation. Smith (4) has drawn attention to the effect of fragmentation on the moisture equivalent determination of heavy soils.

The dense Bale gravelly loam and the primary chaparral soil as they occur in the field are examples of soils that have no permanent wilting percentage that can be determined with undisturbed samples. The difficulty in obtaining reliable information of soil-moisture conditions in such compact soils, by means of instruments that have to be inserted in place at the various depths to be studied, is clear when we take into consideration the fact that the compactness of the soil is changed when the instrument is placed in position. It is possible to remove water from soil by physical means, or to measure its electrical properties, but if the plant cannot remove the moisture because of compactness and the small size of pores, the values obtained are meaningless from an agricultural viewpoint.

#### SUMMARY

Lack of extraction of water from a compact subsoil of Bale gravelly loam in a vineyard and of a primary chaparral soil in an area of native vegetation indicated an absence of roots. Soil samples compacted mechanically to about the same apparent specific gravity that existed in the field were not entered by sunflower

roots. Furthermore, the moisture contents of the compacted samples were about the same at the end of the experiment as at the beginning, showing that there was no moisture extraction by the roots.

The evidence obtained from grapevines in the field and from sunflowers in laboratory trials indicates that roots of these plants were unable to penetrate the Bale soil at an apparent specific gravity of 1.8 or above.

Moisture equivalents of the Bale soil having a density of about 1.8 proved to be an unreliable measure of the field capacity. The definition of field capacity as the amount of water found in a pervious soil 2 or 3 days after a rain or an irrigation is not applicable to Bale gravelly loam, as it takes this soil about 5 months to drain.

Attention is directed to the fact that Bale gravelly loam and the primary chaparral soil are not classed as hardpan soils, having a compact subsoil ordinarily found as a distinct layer, but represent soils having a subsoil that gradually increases in density with depth. Roots in these soils apparently penetrate to a depth at which the specific gravity is about 1.8. Density alone cannot be taken as the limiting value of the ability of roots to penetrate a soil; the size of the individual pores may be the determining factor.

These results indicate the importance of compactness and size of pores as factors tending to accentuate the discrepancies between field and laboratory determinations of the moisture properties of certain soils.

Physical or biological determinations of moisture-holding properties of soils may be unreliable indexes of field-moisture conditions for highly compacted soils. In fact, it may be impossible to obtain such determinations by usual biological means. On the other hand, physical measurements give readings that may be incorrect because the soil is disturbed in making the reading or inserting the apparatus.

#### REFERENCES

- (1) BOYOUKOS, A. J. 1936 Directions for making mechanical analyses of soils. *Soil Sci.* 42: 225-229.
- (2) CARPENTER, E. J., AND COSBY, S. W. 1938 Soil survey of the Napa area, California. U. S. Bur. Chem. and Soils, Soil Surveys, Ser. 1933, No. 13.
- (3) HENDRICKSON, A. H., AND VEIHMEYER, F. J. 1945 Permanent wilting percentages of soils obtained from field and laboratory trials. *Plant Physiol.* 20: 517-539.
- (4) SMITH, W. O. 1944 The effect of soil physical conditions on moisture constants in the upper capillary range. *Soil Sci.* 58: 1-16.
- (5) VEIHMEYER, F. J., AND HENDRICKSON, A. H. 1931 The moisture equivalent as a measure of the field capacity of soil. *Soil Sci.* 32: 181-193.
- (6) WORK, R. A., AND LEWIS, M. R. 1934 Moisture equivalent, field capacity, and permanent wilting percentage and their ratios in heavy soils. *Agr. Engin.* 15: 1-20.

# CHEMICAL CHARACTERISTICS OF HIDALGO FINE SANDY LOAM<sup>1</sup>

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The fine sandy loam of the Hidalgo series is one of the most important soils of southern Texas. In Hidalgo County alone, it covers an area of 106,752 acres which comprises 12 per cent of the total area of this county. Comparative pot experiments carried out by Fraps (9) indicated that Hidalgo fine sandy loam is one of the most productive soils in the Lower Rio Grande Valley. Almost the entire area of this soil type has been brought under cultivation within recent years. The most important crops are citrus, vegetables, and cotton.

The Hidalgo series is a group of upland soils which occupy the southern central part of the Hebbronville Plain as described by Trowbridge (20), or the Beaumont Terrace as described by Foscue (6) and by Wynd (25). Wynd (25, 26) has reported that these soils have developed more or less *in situ*, and they do not represent fluviatile deposits of the Rio Grande. The topsoil becomes progressively more sandy northward because of admixture with wind-blown sea sand.

The parent material is the Beaumont clay formation of the Pleistocene series. This formation was named by Hayes and Kennedy (12) for the exposures near Beaumont, Jefferson County, Texas. Previously, these deposits had been called *Port Hudson* by Hilgard (13) and by Loughridge (16). Dumble (5) referred to them as *coastal clays*. Strictly speaking, the Beaumont clay lies unconformably on the Lissie formations which were previously called *Columbia sands* and are overlain unconformably by the Port Hudson or other fluviatile or wind-blown deposits of the Recent series.

In Hidalgo County, the Beaumont clay lies on the Lissie gravel and is overlain with wind-blown sand and silt, or by fluviatile sand, silt, or clay. In the extreme southern parts of Texas, the Beaumont clay dips below the younger clays and sand which form the present delta of the Rio Grande.

Sellards, Adkins, and Plummer (19) and Trowbridge (20) described these Pleistocene sediments as being mostly laid down by rivers in the form of natural levees and deltas which gradually coalesced by the shifting of the river mouths along the coast, and also as partly deposited by marine and lagoonal waters between the stream ridges and deltas. The shifting of the river mouths caused interbedding of the deltaic, marine, and lagoonal beds. The strata are generally from 400 to 900 feet thick. They are blue, red, or yellowish, and the marine components are usually highly calcareous.

<sup>1</sup> The expenses incurred in the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

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Ries (18) reported the chemical composition of several typical samples of Beaumont clay from Harris County. The samples characterized by their low lime and comparatively high silica content represent nonmarine deposits. The marine deposits are notably richer in lime. The influence of the chemical composition of the parent materials on the character of the soils in regions as young geologically as the Rio Grande Valley is very apparent, and for this reason the data of Ries (18) are briefly summarized as follows:

	HOUSTON	HARRISBURG	CEDAR BAYOU
	per cent	per cent	per cent
SiO <sub>2</sub> .....	89.00	80.94	85.60
Al <sub>2</sub> O <sub>3</sub> .....	3.69	8.09	6.71
Fe <sub>2</sub> O <sub>3</sub> .....	1.65	2.25	1.44
CaO.....	0.47	1.44	Trace
MgO.....	0.65	0.26	0.43
Na <sub>2</sub> O.....	0.06	0.10	0.65
K <sub>2</sub> O.....	Trace	Trace	0.50
TiO <sub>2</sub> .....	0.84	0.78	1.00
H <sub>2</sub> O.....	1.62	6.00	3.10
Total.....	97.98	99.86	99.43

The soils developing from these sediments in the Hidalgo County area are dark gray or dark brown clay loams, overlain in their undisturbed condition by a thin layer of drifting sand. The rainfall is less than 22 inches a year (7), which is not adequate to leach the carbonates completely from the topsoil except where drainage is exceptionally good (25). The original sparse grassland vegetation caused the development of the brown topsoil.

Hawker, Beck, and Devereux (11) described the surface soil of Hidalgo fine sandy loam as brown or light brown calcareous, fine sandy loam, 8 to 12 inches thick. The next layer is brown fine sandy loam or fine sandy clay loam. At depths of 24 to 42 inches, the profile grades into pale yellow, cream-colored, or yellowish fine sandy clay loam, which is usually highly calcareous because of the chemical nature of the parent material and also because of accumulations from the surface layers by leaching. Rarely, local areas contain less than 1 per cent carbonate in the topsoil. The Hidalgo series grades northward into the Brennan series by a lessening of the carbonate in the topsoil due to better drainage. Eastward, it grades into the Victoria series by developing a darker color caused by the increased amount of organic matter which is due to the greater rainfall. Southward, it abruptly borders the low-lying Harlingen series which is composed of recent deltaic clay and silt deposits of the Rio Grande.

Despite the great agricultural importance of Hidalgo fine sandy loam, particularly with reference to the citrus industry, only a very few and incomplete chemical studies of its characteristics have been carried out. Fraps (8) reported the average content of phosphoric acid, nitrogen, potash, lime, magnesium, sulphate, aluminum and iron oxides, and silica in six soils of Hidalgo County.

Undoubtedly the Hidalgo series was included, although the locations and the properties of the samples were not described with sufficient detail to permit them to be referred to any of the soil series described by later authors. After the publication of the detailed soil survey of the region by Hawker, Beck, and Devereux (11), another publication by Fraps (9) appeared in which the nitrogen, total phosphoric acid, active phosphoric acid, total potash, acid-soluble potash, active potash, lime, total basicity, and pH were reported for individual soil types and also as averages for the light-colored and dark-colored soils of the area. The wide variation in the properties of soils studied makes these averaged values of little value in interpreting the characteristics of individual types. This publication, however, is noteworthy for the data reported on the response of Hidalgo fine sandy loam to fertilizer. In pot studies cotton responded favorably to the addition of phosphate and nitrogen, but additional potash was without effect. Another set of similar data was published by Fraps and Fudge (10).

A summary of these previously published analyses appears in table 1.

TABLE 1  
*Summary of previously published analyses of Hidalgo fine sandy loam\**

AUTHOR	NITRO-	TOTAL	ACTIVE	TOTAL	ACID-	Active	ACID-	TOTAL	pH	MgO
	GEN	P <sub>2</sub> O <sub>5</sub>	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	SOLUBLE	K <sub>2</sub> O	SOLUBLE	BASICITY		per cent
1. Fraps (9) .....	0.102	0.089	189	1.52	0.73	764	2.88	4.58	7.2	....
2. Fraps (9).....	0.084	0.056	131	1.74	0.58	270	0.99	1.78	8.1	....
3. Fraps (9).....	0.068	0.063	168	1.62	0.59	279	3.52	5.95	8.3	....
4. Fraps and Fudge (10)	0.066	0.053	60	1.16	0.40	396	4.20	7.46	7.7	0.36
5. Fraps and Fudge (10)	0.059	0.055	70	1.41	0.42	300	5.86	10.68	5.3	0.52

\* See text for explanation.

"Active" phosphorus and potash is the fraction soluble in 0.2 N nitric acid. "Acid-soluble lime" is the amount soluble in strong hydrochloric acid. "Basicity" is the total carbonate content determined by titrating with 0.2 N nitric acid. It includes also about 86 per cent of the exchangeable bases. The set of data numbered 1 represents the average of the dark colored soils of the county reported by Fraps (9). The Hidalgo series is one of the most important of these, although Victoria, Willacy, and Tiocano are also included. Data numbered 2 and 3 are also from Fraps (9) but represent two specific samples of Hidalgo fine sandy loam. Data numbered 4 were published by Fraps and Fudge (10) and are the averaged values of three samples of topsoil of this soil type. The fifth series of data is averaged from three samples of topsoil as reported by Fraps and Fudge (10).

The purpose of the present study was to investigate the chemical properties of a typical profile of Hidalgo fine sandy loam which are of especial importance in the nutrition of plants, and also to determine by comparison, if possible, the chemical peculiarities of sites of different agricultural productivities.

Analyses of two profiles of Hidalgo fine sandy loam are reported. The samples were collected on the Evergreen Farms in the vicinity of Elsa, Texas. Profile 1 is a typical productive representative of this soil type as determined by yields of alfalfa, rye, and Sudan grass over a period of several years. Profile 2 represents a less productive area. Both sites are in Lot 14 of Block 94 in the Missouri-Texas Land and Irrigation Company Subdivision of the Las Mestanas Grant, Hidalgo County.

#### METHODS AND RESULTS

*Calcium carbonate.* The total carbonate was calculated as the calcium salt from the weight of carbon dioxide evolved from the sample when it was treated with 10 per cent hydrochloric acid containing 5 per cent stannous chloride ac-

TABLE 2  
*Chemical properties of Hidalgo fine sandy loam*

DEPTH OF SAMPLE	CaCO <sub>3</sub>		BICARBONATE		pH		NITROGEN		LOSS ON IGNITION		BASE-EXCHANGE CAPACITY		BASE SATURATION*	
	1†	2‡	1	2	1	2	1	2	1	2	1	2	1	2
	inches	per cent	per cent	m.e.§	m.e.§		per cent	per cent	per cent	per cent	m.e.§	m.e.§	per cent	per cent
0-6	1.39	0.65	1.05	0.88	8.2	7.4	0.093	0.090	2.80	2.98	14.5	15.5	273	201
6-12	2.82	2.34	0.80	0.75	8.2	7.6	0.078	0.075	2.72	3.21	13.8	15.7	358	306
12-18	6.55	3.86	0.66	0.76	8.2	7.6	0.059	0.056	2.71	3.01	13.8	15.2	488	405
18-24	10.3	9.85	0.74	0.67	8.2	7.9	0.046	0.049	2.39	2.93	13.4	15.0	530	490
24-30	13.8	14.7	0.70	0.64	8.2	8.0	0.042	0.046	2.03	2.91	12.9	15.2	602	490
30-36	18.7	17.9	0.70	0.70	8.2	8.2	0.039	0.043	1.79	2.55	12.7	15.2	640	524
36-42	21.1	18.9	0.62	0.70	8.3	8.2	0.033	0.036	1.54	2.40	12.2	15.3	659	508

\* Data calculated on the basis of the ammonium acetate leachate. See text for further details.

† 1 = profile from a productive area.

‡ 2 = profile from a less productive area.

§ Per 100 gm. of oven-dry soil.

cording to the A. O. A. C. procedure (1). Data in table 2 indicate that the amount of calcium carbonate increased rapidly with depth. At 24 inches the soil was about 10 per cent calcium carbonate, and at 42 inches, about 20 per cent. This increase occurred at an approximately even rate in the better soil. In the poorer soil, the amount increased abruptly from 3.86 per cent in the 12- to 18-inch layer to about 10 per cent in the 18- to 24-inch layer.

*Bicarbonate.* The bicarbonate was determined by the micromethod described by Reitemeier (17), which is based on the procedure originally published by Warder (22). A sample of 50 gm. of dry soil was shaken with 250 ml. of water for 1 hour and then allowed to stand 24 hours. Aliquots of 25 ml. of the supernatant liquid were titrated with 0.01 N sulfuric acid, with methyl orange as indicator. Phenolphthalein was also added to each aliquot before titration. The absence of a pink color indicated the absence of soluble carbonates in all of the samples studied.

The data in table 2 show the presence of considerable bicarbonate in the upper 12 inches of soil, although the poorer soil contained less bicarbonate than the productive soil. This situation, together with the pH values presented in table 2, makes it apparent that the poorer soil did not owe its low productivity to "black alkali." There was a decreasing amount of bicarbonate with depth. Below 12 inches, the amount was very small.

*pH.* The pH was determined colorimetrically in the centrifuged supernatant layer of the water extract. The data in table 2 show that the pH of the better soil was consistently 8.2 to a depth of 36 inches, with a slight increase at 42 inches, whereas that of the poorer soil increased from 7.4 in the surface layer to 8.2 at the lower levels. The more acid condition of the unproductive soil was probably dependent on the larger amount of organic matter and the consequent smaller degree of base saturation.

*Nitrogen.* Total nitrogen was determined according to the A. O. A. C. procedure (1). The data (table 2) indicate that the amount of nitrogen decreased rapidly from about 0.09 per cent at the surface to about one half this value at 24 inches. Below this depth, nitrogen continued to diminish, but at a decreasing rate.

*Loss on ignition.* The loss on ignition was determined by the A. O. A. C. method (1). The ash was treated with saturated ammonium carbonate and dried at 200° C. so that all oxides were converted to carbonates. The data in table 2 show a progressively smaller amount of organic matter with increasing depth. The greater amount of organic matter in the poorer soil seems inconsistent with its poor quality. It is possible that this condition reflects poor drainage and an unsatisfactory bacterial activity in the poorer soil.

*Base-exchange capacity.* The base-exchange capacity was determined by titration according to the method of Bray and Willhite (3) and Bray<sup>3</sup>. Table 2 shows that the base-exchange capacity decreased with depth in the good soil but was higher and more constant in the poorer soil. The data show that this property depends largely on the amount of organic matter. The clay component of the soil, especially at the lower levels, also contributes significantly.

*Total replaceable bases.* The total replaceable bases were determined by the ammonium acetate procedure described by Bray and Willhite (3) and Bray<sup>3</sup>, and also by the sodium acetate procedure of Hissink (14).

Table 3 shows that the quantity of total replaceable bases, determined in the ammonium acetate leachate, increased rapidly with depth. In comparison with the surface soil, the amount about doubled at the 18-inch level. Since many salts are more soluble in solutions of ammonium acetate than in pure water, it follows that these determinations do not represent strictly "replaceable" bases. They do, however, show that the soil contained large stores of plant nutrients and that the amounts increased with depth. The data in table 3 show that this increase was due almost exclusively to calcium.

A very different result was obtained when the procedure of Hissink (14), which

<sup>3</sup> Bray, R. H. Base exchange procedures. Ill. Agr. Exp. Sta., Dept. Agron., Mimeo. Bul. Ag. 1010. 1942.

is especially designed for the analysis of calcareous soils, was used. The data in table 4 show that the total replaceable bases decreased slightly with increasing depth in the profile. It is especially interesting to note that the poorer soil contained significantly greater quantities than the productive soil.

*Base saturation.* The degree of base saturation, as determined by the ammonium acetate leachate procedure, increased rapidly with depth. Since base-exchange capacity decreased with depth, and the total replaceable bases

TABLE 3

*Replaceable bases in Hidalgo fine sandy loam, determined in the ammonium acetate leachate*

DEPTH OF SAMPLE	TOTAL REPLACEABLE BASES		Ca		Mg		K		Na	
	1*	2	1	2	1	2	1	2	1	2
inches	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.
0-6	38.8	31.0	34.8	27.8	4.35	3.66	1.27	0.99	1.50	2.16
6-12	50.7	48.0	49.0	48.2	4.15	3.30	1.17	1.07	0.50	0.41
12-18	67.3	61.5	57.3	59.5	3.32	2.85	0.34	0.29	0.27	0.50
18-24	70.7	73.5	63.5	71.7	2.80	3.80	0.23	0.22	0.38	0.46
24-30	77.8	74.8	73.7	77.1	2.70	4.60	0.23	0.15	0.23	0.39
30-36	81.5	79.5	74.5	75.0	3.12	4.83	0.22	0.22	0.25	0.52
36-42	80.3	77.8	76.1	70.5	3.68	4.80	0.18	0.15	0.21	0.59

\* See footnotes to table 2.

TABLE 4

*Replaceable bases in Hidalgo fine sandy loam, determined by the method of Hissink for calcareous soils*

DEPTH OF SAMPLE	TOTAL REPLACEABLE BASES*		REPLACEABLE CALCIUM		REPLACEABLE MAGNESIUM	
	1†	2	1	2	1	2
inches	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.
0-6	13.79	17.98	9.27	12.51	1.75	2.32
6-12	13.56	16.86	10.07	13.28	1.82	2.10
12-18	14.04	16.54	11.68	13.51	1.75	2.24
18-24	13.04	16.33	10.77	13.24	1.56	2.41
24-30	12.85	15.10	10.73	12.07	1.66	2.49
30-36	12.18	16.18	9.95	12.54	1.76	2.90
36-42	12.23	14.60	10.04	11.09	1.80	2.77

\* Sum of replaceable Ca, Mg, K, and Na.

† See footnotes to table 2.

increased, the percentage base saturation increased rapidly. The high values presented in table 2 show that only one half to one eighth of the bases leached by neutral normal ammonium acetate were actually removed from the soil colloids. It does not follow, however, that these excess salts are necessarily water-soluble, since many salts in the soil are more soluble in the acetate solution than in pure water. It would be more correct to assume that all samples were 100 per cent saturated, since the soils were calcareous.

*Replaceable calcium.* Replaceable calcium was determined in the ammonium acetate leachate by titrating the oxalate precipitate with cerate as described by Bray<sup>3</sup>. Data in table 3 show that the amount of replaceable calcium, plus that soluble in ammonium acetate, increased rapidly with depth. The poorer soil showed a zone of accumulation at a depth of 24 to 30 inches.

When the more appropriate method of Hissink was used, the amount of replaceable calcium did not vary significantly with increasing depth in the profile (table 4).

*Replaceable magnesium.* Replaceable magnesium was determined in the ammonium acetate leachate by precipitation with 8-hydroxy quinoline as described by Bray<sup>3</sup>. Both profiles exhibited a zone of lessened replaceable magnesium (table 3). This occurred at about 18 inches in the poorer soil, and at about 30 inches in the better soil. Below 18 inches there was a rapid increase in the poorer soil, and a smaller increase in the better soil.

The data in table 4, obtained by Hissink's procedure, show that the amounts of replaceable magnesium did not vary significantly with depth in the better soil and increased slightly with depth in the poorer soil.

*Replaceable potassium.* Replaceable potassium was determined by precipitation by cobaltinitrite as described by Wilcox (23) and then determined colorimetrically, by the procedure of Wander (21). The data in table 3 indicate that the upper 12 inches of soil contained comparatively large amounts of replaceable potassium. Below this level, the amount was small and remained comparatively constant. This suggests that the high solubility of its salts has enabled a generally upward migration during periods of exceptionally moist conditions with a consequent concentration at the surface by the evaporation of soil moisture. Both soils were remarkably similar in respect to the distribution of replaceable potassium in the profile.

*Replaceable sodium.* Replaceable sodium was determined by the procedure of Barber and Kolthoff (2). Data in Table 3 show that replaceable sodium, like replaceable potassium, was concentrated at the surface, and probably the same factors have been effective in accounting for this situation. Both profiles exhibit a similar distribution of replaceable sodium, although the poorer soil contained slightly more, especially at the lower levels.

*Total water-soluble salts.* The total water-soluble salts were determined by the procedure recommended by the A. O. A. C. (1). Suitable aliquots of the water extract were used to determine the individual water-soluble components. Table 5 shows that the quantity of water-soluble salts decreased rapidly with depth in the better soil. In the poorer soil, a zone of accumulatoin appeared in the 24-to-30-inch layer. The data indicate that calcium sulfate was the chief component of the water-soluble salts in this layer. It does not follow necessarily that all of the calcium sulfate recovered from the water extract was originally present in the dissolved condition, since the solubility of calcium sulfate in the water used for leaching is 0.241 gm. per 100 ml. at 0°C., which corresponds to 558 p.p.m. of calcium. This makes it evident that considerable amounts of calcium sulfate can be removed by water extraction. It should be borne in mind also

that water extracts are particularly difficult to free from suspended, finely divided calcium sulfate crystals without simultaneous removal of ions from the solution.

*Water-soluble sodium.* Water-soluble sodium decreased with depth. It is evident from comparison with the total water-soluble salts that the zone of apparent soluble salt accumulation in the poorer soil was not due to this ion.

*Water-soluble calcium.* The data in table 5 show that water-soluble calcium decreased gradually with depth in the good soil, but a zone of accumulation appeared in the poor soil in the 24- to 30-inch layer. This corresponds with the zone of total apparent soluble salt accumulation. Comparison with the data in table 4 shows that large amounts of calcium were dissolved by 1 N ammonium acetate and that this amount increased with depth while the amount soluble in pure water decreased with depth. The amount soluble in ammonium acetate increased as the amount of calcium carbonate increased, a relationship not true for water-soluble calcium.

*Water-soluble magnesium.* The data for water-soluble magnesium, presented

TABLE 5  
Water-soluble components in Hildalgo fine sandy loam

DEPTH OF SAMPLE	TOTAL		Na		Ca		Mg		SO <sub>4</sub>		PO <sub>4</sub>		Cl	
	1°		2		1		2		1		2		1	
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.								
inches														
0- 6	624	537	70	99	340	310	10	9	161	101	0.388	0.469	35	19
6-12	638	579	67	81	318	310	3	19	194	107	0.347	0.254	65	63
12-18	504	503	52	56	270	300	5	2	134	101	0.153	0.234	49	44
18-24	495	540	50	54	320	330	6	1	87	110	0.122	0.153	33	45
24-30	431	640	49	52	270	410	7	9	86	128	0.132	0.181	21	42
30-36	431	419	39	39	290	250	8	5	78	87	0.092	0.090	17	39
36-42	432	430	45	32	260	270	2	6	105	93	.....	.....	21	37

\* See footnotes to table 2 for identification of 1 and 2.

in table 5, appear at first glance to be irregular, but comparison with table 4 shows interesting relationships. The low amounts of replaceable magnesium in the good soil corresponded to an increased amount of water-soluble magnesium. This was probably due to the rapid decrease in the base-exchange capacity with depth, which allowed the increasing amounts of calcium to replace relatively more of the magnesium on the colloids. In the poor soil, the higher amounts of replaceable magnesium were associated with high amounts of water-soluble magnesium. This suggested that at the lower levels of the profile of the poorer soil, most of the so-called "replaceable" magnesium removed by the ammonium acetate leaching was not attached to the colloids of the soil, but was in a water-soluble, or at least an ammonium-acetate-soluble, form. The increase in the amounts of replaceable magnesium in relation to the amounts of water-soluble magnesium depended on the fact that magnesium was in less competition with calcium for space in the soil colloids because of the greater base-exchange capacity of the poorer soil. It must be borne in mind that the increased amount of apparently replaceable magnesium in the neutral normal

ammonium acetate leachate, as well as that in the water extract, was partly due to the increase of magnesium sulfate, since both magnesium and sulfate had a comparatively high concentration at the same level.

*Water-soluble sulfate.* The sulfate in the water-soluble extract was determined by precipitation as barium sulfate (1). The data in table 5 show that the amount of water-soluble sulfate decreased with depth in the good soil. In the poor soil, the amount was very much less in the upper layer, but an increase occurred in the 24- to 30-inch zone, which corresponded to the zone of general soluble salt accumulation. In fact, this accumulation appeared to be composed largely of calcium sulfate.

*Water-soluble phosphate.* The water-soluble phosphate was determined colorimetrically by the procedure of Dickman and Bray (5). The Kurtz (15) modification was used to prevent the interference of fluoride ions as described by Wynd and Noggle (24). Data presented in table 5 show that the topsoil contained significant amounts of water-soluble phosphate, which decreased rapidly with depth. It is frequently assumed without definite data that phosphate is comparatively unavailable to plants in alkaline soils. The present data, together with other unpublished data obtained by the authors, show that this assumption is quite generally untrue.

*Water-soluble chloride.* The chloride in the water extract was determined as recommended by the A. O. A. C. (1). Data in table 5 show that there was more water-soluble chloride below a depth of 18 inches in the poor soil than in the better soil. The concentration of this ion, however, did not vary according to the total water-soluble salts, and the actual amounts appeared to be too small to account for the poorer productivity of this soil.

#### DISCUSSION OF RESULTS

A comparison of the data presented in the tables indicates that the profile of the poorer soil differed in but few well-marked chemical properties from that of the more productive soil. The poor soil had a conspicuously higher base-exchange capacity due to its greater content of organic matter, a condition which was probably dependent on differences in drainage and consequently also in bacterial activity. The poorer soil also contained greater quantities of replaceable calcium and magnesium.

There was a marked accumulation of water-soluble salts at the 24- to 30-inch level in the poorer soil. This accumulation was due primarily to calcium sulfate. Magnesium also contributed to this zone of soluble-salt accumulation, but to a much less degree than did calcium.

Many agriculturalists in the Rio Grande Valley occasionally plow their fields very deeply because of the general belief that plant nutrients occur in larger amounts in the lower levels of the profile. In exceptional cases, fields have been plowed to a depth of 24 inches in the hope of increasing the productivity of depleted soils. The present study indicates that only ammonium-acetate-replaceable calcium and calcium carbonate increase significantly with depth, and since this nutrient is already present in large excess in these calcareous soils,

the bringing to the surface of additional calcium could scarcely increase the growth of the crop. The favorable influence which deep plowing sometimes exerts on plant growth in this area is probably dependent on its effect on the physical condition of the soil.

#### SUMMARY

The geological and petrographic characteristics of the parent materials of Hidalgo fine sandy loam have been described.

The chemical properties of the profiles of two soil sites of different agricultural productivity, near Elsa, Hidalgo County, Texas, were determined at successive 6-inch levels to a depth of 3.5 feet. The samples were studied in respect to calcium carbonate; nitrogen, base-exchange capacity, total bases replaced and dissolved by normal neutral ammonium acetate; base saturation; loss on ignition; pH; bicarbonate; soluble carbonate; replaceable calcium, magnesium, potassium, and sodium; total water-soluble salts; and water-soluble sodium, calcium, magnesium, sulfate, and chloride.

Comparison of the chemical characteristics of the profiles showed that the poorer site had a higher base-exchange capacity because of its higher content of organic matter. This condition was probably dependent on poorer drainage.

There was a marked accumulation of water-soluble salts at the 24- to 30-inch level in the poorer soil. These salts were primarily calcium sulfate, although significant amounts of magnesium sulfate also were present.

There were more chlorides at the lower levels of the profile of the poorer soil, but the amounts were too small to account by themselves for the lower productivity. There was no zone of chloride accumulation, and its amount decreased rapidly with depth.

Sodium was present in exceptionally small amounts for an alkaline pedocal soil.

Physical conditions of the soil, especially drainage in the lower levels of the profile, appeared to be more significant than chemical properties in governing local variation in agricultural productivity.

The custom of occasional exceptionally deep plowing of depleted soils in the Rio Grande Valley to bring to the surface additional stores of plant nutrients has been deemed unwarranted, since the concentration of all plant nutrients, with the exception of replaceable calcium, has been found to diminish with depth.

#### REFERENCES

- (1) Association of Official Agricultural Chemists 1935 *Official and Tentative Methods of Analysis*, ed. 4. Washington, D. C.
- (2) BARBER, H. H., AND KOLTHOFF, I. M. 1928 A specific reagent for the rapid gravimetric determination of sodium. *Jour. Amer. Chem. Soc.* 50: 1625-1631.
- (3) BRAY, R. H., AND WILLHITE, F. M. 1929 Determination of total replaceable bases in soils. *Indus. and Engin. Chem., Analyt. Ed.* 1: 144.
- (4) DICKMAN, S. R., AND BRAY, R. H. 1940 Colorimetric determination of phosphate. *Indus. and Engin. Chem., Analyt. Ed.* 12: 665-668.
- (5) DUMBLE, E. T. 1894 The Cenozoic deposits of Texas. *Jour. Geol.* 2: 549-567.

- (6) FOSCUE, E. J. 1932 Physiography of the Lower Rio Grande Valley. *Pan-Amer. Geol.* 57: 263-267.
- (7) FOSCUE, E. J. 1932 The climate of the Lower Rio Grande Valley of Texas. *Mo. Weather Rev.* 60: 207-214.
- (8) FRAPS, G. S. 1909 The chemical composition of some soils of Angelina, Brazoria, Cameron, Cherokee, Delta, Lamar, Hidalgo, Lavaca, Montgomery, Nacogdoches, Robertson, Rusk, Webb, and Wilson Counties. *Tex. Agr. Exp. Sta. Bul.* 125: 1-84.
- (9) FRAPS, G. S. 1933 Soils of Henderson, Hidalgo, Milam, Nacogdoches, Navarro, Wichita, Willacy, and Victoria Counties. *Tex. Agr. Exp. Sta. Bul.* 482: 1-64.
- (10) FRAPS, G. S., AND FUDGE, J. F. 1937 Chemical composition of the soils of Texas. *Tex. Agr. Exp. Sta. Bul.* 549: 1-87.
- (11) HAWKER, H. W., BECK, M. W., AND DEVEREUX, R. E. 1925 Soil survey of Hidalgo County, Texas. U. S. Bur. Chem. and Soils, Ser. 1925, No. 13.
- (12) HAYES, C. W., AND KENNEDY, W. 1903 Oil fields of the Texas-Louisiana Gulf Coastal Plain. *U. S. Geol. Survey Bul.* 212: 1-174.
- (13) HILGARD, E. W. 1869 Summary of results of a late geological reconnaissance of Louisiana. *Amer. Jour. Sci.* (2nd Ser.) 48: 331-346.
- (14) HISSINK, B. I. 1923 Method for estimating adsorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269-276.
- (15) KURTZ, L. T. 1942 Elimination of fluoride interference in the molybdenum blue reaction. *Indus. and Engin. Chem., Analyt. Ed.* 14: 855.
- (16) LOUGHBRIDGE, R. H. 1884 Physico-geographical and agricultural features of the State of Texas. *Tenth Census U. S.* 5: 669-806.
- (17) REITEMEIER, R. F. 1943 Semimicroanalysis of saline soil solutions. *Indus. and Engin. Chem., Analyt. Ed.* 15: 393-402.
- (18) RIES, H. 1908 The clays of Texas. *Univ. Texas Bul.* 102 (Sci. Ser. 2, no. 12): 1-316.
- (19) SELLARDS, E. H., ADKINS, W. S., AND PLUMMER, F. B. 1932 The geology of Texas. Vol. 1. Stratigraphy. *Univ. Tex. Bul.* 3232: 1-1007.
- (20) TROWBRIDGE, A. G. 1932 Tertiary and Quaternary geology of the Lower Rio Grande region, Texas. *U. S. Geol. Survey Bul.* 837: 1-280.
- (21) WANDER, I. W. 1942 Photometric determinations of potassium. *Indus. and Engin. Chem., Analyt. Ed.* 14: 471.
- (22) WARDER, R. B. 1881 Alkalimetry with phenol phthalien as indicator. *Chem. News* 43: 228-229.
- (23) WILCOX, L. V. 1937 Determination of potassium by means of an aqueous solution of trisodium cobaltinitrite in the presence of nitric acid. *Indus. and Engin. Chem., Analyt. Ed.* 9: 135-138.
- (24) WYND, F. L., AND NOGGLE, G. R. 1943 Associations between phosphorus fractions and other chemical components in soil. *Soil Sci.* 56: 383-391.
- (25) WYND, F. L. 1944 The soil series represented in Hidalgo County, Texas. *Amer. Midland Nat.* 32: 181-199.
- (26) WYND, F. L. 1944 The geological and physiographic background of the soils in the Lower Rio Grande Valley, Texas. *Amer. Midland Nat.* 32: 200-235.



# MODERN CONCEPTS OF SOIL SCIENCE<sup>1</sup>

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Soil science is concerned with the principles that are involved in the soil, in its formation, transformation, utilization, and conservation—in a word, with soil processes. From whatever point of view the soil is considered, whether purely utilitarian, or as a natural body, the properties of the soil are determined by the forces that are inherent in it.

The soil may be considered from four points of view: (a) its formation, (b) its constituents, (c) the forces that are involved, and (d) as a medium for the growth of plants. The last named is closely associated with the ability of the soil to supply nutrients to growing plants. Since the crop-producing power of the soil depends on the properties of the soil, and the properties depend on the interplay of the forces acting on the constituents of the soil, an understanding of the crop-producing power must rest on an understanding of these forces.

The properties of the soil are largely predetermined by its origin, that is, by the parent material as influenced by the natural conditions to which it has been subjected. Therefore, an understanding of both the formation of and the forces inherent in the soil are fundamental to an understanding of soil fertility. Researches on soils have sought to throw light on these questions.

Present-day views of the soil may be summarized under five headings:

1. *The concept of ion exchange, both cation and anion.* Much of the chemistry of the weathering of primary minerals involves an exchange of cations. Likewise, a high percentage of the chemical reactions taking place naturally or produced artificially in soils also involve ion exchange. The clay and humus constituents are the components of the soil most largely involved in the exchange process.

2. *The dynamic concept.* The soil is a highly dynamic system. It responds readily to chemical, physical, and biological forces, and it is teeming with living organisms. These organisms effect deep-seated chemical and physical changes, not alone in the organic substances but in the inorganic as well.

3. *The concept of potential.* Both the physical and chemical potentials of the components of the soil are important. The movement of water in the soil is now recognized as being largely controlled by potential, gravitational potential as regards the movement of free water, capillary functions as regards thin films of water. These forces are now being expressed by soil physicists in precise physical terms. The chemical potential of the ions on the surface of particles largely determines the reactions which take place at the surface, and it is highly probable

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that the activity of exchangeable ions differs from that of solution ions. There is great need for better understanding of the chemical and electrical forces at the interphase between solid particles of different kinds and solution films.

4. *The concept of development.* The soil is distinctly not a mere heterogeneous collection of rock fragments, disintegration products of weathering, and organic matter. The soil is not mere dirt. Elimination of soluble products, segregation and concentration of others, and sometimes intensive weathering at certain depths, due to moisture distribution, have brought about the development of what is called "the soil profile." The degree of development is extremely varied, but, excepting very recently deposited material, a certain degree of development is found in all soils. The profile is characterized by a sequence of horizons. The nature of these horizons may vary greatly from soil to soil. The profile as a whole must be given due consideration in the interpretation of the properties of any one of the horizons.

5. *The plant nutrient concept.* From early days many soil investigators have centered their attention on nitrogen, phosphorus, and potassium and on methods of determining the so-called availability of these elements; soil fertility is often defined in terms of these three elements. However, the crop-producing power of the soil involves far more than nitrogen, phosphorus, and potassium. The idea that nitrogen, phosphorus, and potassium are the only elements that are likely to be deficient has been thoroughly discredited. Calcium, magnesium, sulfur, among the so-called major elements, and copper, zinc, boron, manganese, molybdenum, cobalt, iodine, and perhaps others required in minute amounts may also be deficient either from the standpoint of plant growth or that of the well-being of the animals that consume the plant. Many of these elements are held on the surface of clay particles by electrostatic attraction. Different types of clay are characterized by differences in the strengths of this attraction, and by the amount of the attracted ions. For these reasons the type of clay present has an important bearing on plant nutrition.

#### CLAY

During the last two decades there has been a steadily increasing recognition of the part played by the clay constituents of the soil. Growing out of the researches on clay, it has become apparent, though not fully appreciated by all soil workers, that an understanding of a number of soil properties depends on an understanding of clay itself. In a large though not all-inclusive sense, the modern concept of the soil can then be said to be the concept of clay.

Clay is important not alone because of its role in cultivation, crop adaptation, and soil classification. It is the key to an important part of the chemistry and physics of the soil, to base exchange, to soil morphology, and to plant nutrition. The force of this fact has come to be realized in increasing measure in recent years. Not more than a mere outline of the researches on clay can be given here.

In contrast to the view held throughout the greater part of the history of soil investigation, namely, that the finely divided inorganic material of soils is amorphous, and that it consists chiefly of an indefinite mixture of the oxides of silicon,

aluminum, and iron in varying stages of hydration, we now know that the clay material is neither amorphous nor primarily a mixture of uncombined oxides. Rather it is composed largely of clay minerals of definite mineralogical characteristics. The kind of clay minerals, as well as the amounts present, largely determine the properties of the soil. Thus the quality of the clay is an important consideration.

Formerly base exchange, or more properly cation exchange, was thought of as being an aspect of the colloidal nature of the soil material. Expressions such as "the colloidal complex" and "the colloidal adsorption complex" have been and still are widely used in soil literature. Wiegner (5) suggested that the electro-negative character of colloids, including the fine-grained material of soils, is due to the adsorption of OH ions. In consequence of the adsorbed OH ions, cations are also adsorbed, and these in turn are replaceable. Researches based on recently developed methods have invalidated this view completely.

Soil clay is now known to be definitely crystalline, and the crystal structure of the few kinds of clay minerals found in soils has been determined. Certain of these have marked cation-exchange power, others very little exchange capacity. The composition and arrangement of the atoms comprising the lattice structure of the clay minerals are such as to require the presence of external cations in order to balance the charge of the lattice (1) and some, at least, of these cations are exchangeable. The cations are held largely by electrostatic attraction rather than by covalent bonds, but these cations are essential parts of the crystal. They tend, however, to dissociate in an aqueous medium and in consequence the particles act as if negatively charged. In a dry state, the particles are no more charged than are neutral molecules of NaCl or any other chemical compound.

Furthermore, we can say that, apart from organic substances, the base-exchange property of soils is generally occasioned by the clay minerals present. Thus the notion that the soil contains zeolitic material, which was formerly the prevailing view, is no longer tenable. Our present understanding of the clay minerals and how they contribute to soil properties has been obtained by the application of established principles of chemistry and mineralogy.

#### *Soil acidity in relation to clay*

When a soil is treated with a mild chemical reagent, it is the clay that reacts most readily. This is because the clay contains exchangeable cations. These cations can be reversibly replaced by a great variety of other cations. Under protracted leaching with rain water, not only are soluble substances leached out, but also the H ions of the water gradually replace metallic cations from the surface of the clay minerals, and this results in the clay's becoming definitely acidic. The soil is acidic because the clay contains exchangeable H ions. However, it seems necessary to emphasize that whatever the exchangeable cation, whether the Ca, Mg, K, Na, or H ion, cations in addition to those of the framework of the lattice are essential to the integrity of the clay mineral.

It follows then that acid soils do not necessarily contain soluble acids. Just as base exchange derives mainly from the clay minerals, soil acidity is likewise a

property of clay. It is a manifestation of cation exchange. Cation exchange is indeed an important property of clay.

#### *Clay in relation to the growth of plants*

Clay, by virtue of its exchangeable cations, also plays an important part in the nutrition of plants. Experiment has shown that certain cations, held in exchangeable form by clay, can be taken up by plant roots, and possibly without passing through an intermediate solution state (2). Thus, insofar as the major cations are concerned, the growing plant can draw directly on the supply residing in the clay minerals. The plant is therefore not solely dependent on soluble salts. This, of course, should not be interpreted to mean that the soluble constituents of soils are unimportant. However, in the absence of insoluble carbonates, the cations, found in soils as soluble salts, have in large part been derived from exchangeable forms and have been brought into solution by exchange with H ions of biological origin.

Clay also has an important bearing on the uptake of anions by plant roots. Phosphate adsorbed on clay particles seems to be especially important in acid soils and is brought into solution or is absorbed by plant roots directly through the process of anion exchange. It is probable that certain of the minor elements are also largely adsorbed on clay as anions. To a very large extent soil chemistry is the chemistry of ion exchange.

#### *Calcium carbonate in relation to clay*

Under the influence of leaching and the growth of plants, especially in humid climates where leaching is relatively active, the soil, as is well known, tends to become acidic. This, however, cannot amount to much as long as the soil contains calcium carbonate, because calcium carbonate reacts readily with clay whenever it contains exchangeable H ions. The result is that H ions are replaced by Ca ions and then the clay is no longer acid. Calcium carbonate keeps the clay minerals largely Ca-saturated despite leaching. Ca-saturated minerals are neutral or only mildly alkaline.

Calcium carbonate performs two other functions, both of which have to do with the clay minerals.

First, by virtue of maintaining a state of high Ca saturation, the chemical stability of clay minerals is promoted. When H ions constitute a high percentage of the total exchangeable cations, that is, when the soil is largely unsaturated with bases, certain clay minerals tend to decompose. There is some evidence that soil clay high in cation-exchange capacity tends, under certain conditions, as when it is subjected to prolonged leaching, to become low in cation-exchange capacity (3). For example, upon repeated desaturation followed by saturation with Ca ions on Ba ions, the base-exchange capacity of montmorillonite has been found to decrease with each repetition of the process. This indicates that the concentration of H ions required to effect desaturation slowly decomposes the clay. When the soil becomes strongly acidic, it appears that montmorillonite,

if present, gradually passes into kaolinite, which is low in base-exchange capacity. Furthermore, well-drained soils of humid climates are generally kaolinitic. Thus it seems that the preservation of high absorptive power in the soil is conditioned in part at least on the avoidance of the development of strong acidity.

The second reason why calcium carbonate is important is that calcium-saturated clay tends to be well granulated and this promotes good tilth. Ca-saturated clay resists dispersion and hence it operates to prevent the formation of dense clay horizons at some distance below the surface. Thus the clay minerals are directly involved in morphological differentiation in the soil profile.

#### *Alkali soils in relation to clay*

Where sodium salts accumulate, as in dry climates, Na ions replace Ca ions from the clay with the formation of sodium clay. This leads to very important alterations in the chemistry, physics, and morphology of the soil and to extremely adverse conditions for plant growth. Sodium clay tends to hydrolyze with the formation of NaOH. Thus soils high in replaceable sodium tend to be alkaline. Sodium clay also disperses very readily. This adversely affects the permeability of the soil to air and water. Only a relatively low degree of sodium saturation of clay lowers its permeability to water. This is the chief reason why saline irrigation waters that are relatively high in sodium should be avoided. By the use of such waters, the soil may acquire enough exchangeable sodium to affect its permeability seriously. This effect may be masked by the flocculation action of soluble electrolytes, but upon leaching out the soluble salts, it becomes pronounced.

An alkali soil, then, is not necessarily one that merely contains an excess of soluble salts. The clay itself, under certain conditions, becomes profoundly altered by the soluble salts. Therefore the reclamation of an alkali soil is not always restricted to the removal of soluble salts. The clay itself may also need be changed. A large part of the sodium held by the clay must be replaced by calcium before the soil will be fully reclaimed. By taking advantage of the principles worked out in various laboratories, methods are now available by which large areas of unproductive alkaline woils can be converted into highly productive soils.

The researches in this field, incidentally, afford a striking example of the practical value of scientific research. Before the cation-exchange relationships of soils were understood, the reclamation of vast areas of alkali soils was extremely difficult. Another point of very great practical value has come to light as an outgrowth of research of alkali soils, namely, that the permanence of irrigation soils is not necessarily short-lived, as has so often been averred.

#### *Clay in relation to the physical property of soils*

Modern researches on the clays have shown that these minerals are platy. Anything that promotes dispersion of the soil, that is the breaking up of ag-

gregates, accentuates the effects of the platiness of the particles. Certain well-recognized physical forms among the structural units of soils owe their very existence to the fact that the clay particles are platy. Soil morphology is therefore related to clay.

#### *Clay in relation to the buffer properties of the soil*

As has been pointed out, H ions readily replace metallic cations from clay with a resulting increase in the acidity of the clay. However, the dissociation of the H ions on clay is probably never complete. In effect, the clay acts as a buffer against extremes of acidity. A considerable part of the metallic cations must be replaced by H ions before the pH becomes less than 6.0. This property of clay serves to retard the development of excessive acidity that would otherwise result from the accumulation of biologically formed acids.

Thus we can say that the clay is one of the most important constituents of the soil. Further, the better clay itself is understood, the more definite will be the understanding of soil properties. The significance of the properties of the clay minerals in their bearings on the chemical and physical properties of the soil has not been sufficiently well recognized by soil workers.

As was indicated earlier, different kinds of soils contain different kinds of clay minerals. The crop-producing power of the soil, however, is not necessarily correlated with a single kind of clay mineral. It is true, large areas of highly fertile soils are characterized by one kind of clay, the montmorillonitic type, but certain areas where this type of clay predominates are very far from ideal soils, for example, the Putnam soils of the lower Missouri Valley, certain adobe-like types in other places, and perhaps more striking still, certain soils derived from serpentine rocks. Neither is soil acidity dependent on one kind of clay. Any kind of clay can be made distinctly acid by replacement of its exchangeable metallic cations by H ions.

From the foregoing it follows that soil chemistry is largely concerned with chemistry at interphases, that is, reactions at the surface of soil particles. Soil chemistry is indeed an excellent example of surface chemistry. In other words, the forces on the surfaces of soil particles determine the reactions that take place. Therefore, an understanding of the nature of these forces is fundamental to an understanding of the soil and of its properties. It would be difficult to exaggerate the importance of this point, for without an understanding of the forces that are involved in a process, the process itself cannot be fully understood. This fact applies to soil physics as well as to soil chemistry. That surface forces are involved in the water relationships of soil is certain, and they are undoubtedly involved in soil granulation and tilth. The chemical potential at the interphase likewise determines the chemical reactions that take place. The chemistry of the clay surfaces is very complex. Certain soil scientists hold that the reactions at the interphase can not be fully explained on the basis of ordinary chemical concepts.

In the foregoing no attempt has been made to give a comprehensive review of present-day soil science. Whole fields of the subject have been by-passed.

In fact, only one phase has been emphasized. Significant advances have, however, been made on other aspects of soil science.

#### CALCIUM

Perhaps it is permissible to allude briefly to another point. Running through the researches on soils like a silver cord is the element calcium. This element takes part in many soil processes, and among all the elements, calcium, by virtue of the multiplicity of processes in which it takes part, is undoubtedly the most important. This element in addition to many others is essential to plant growth; the lack of calcium is responsible for the development of soil acidity. In another sense, the same is true of alkali soils, although in this case it is merely the clay that is deficient in calcium. In contrast to acid soils, alkali soils usually contain an abundance of calcium carbonate. Calcium markedly influences the physical properties of the soil, and in the form of plant substance, calcium, derived from the soil, plays a very great role in animal and human nutrition. In humid climates, soils, said to be worn out, are likely to be deficient in calcium.

However, calcium need not be expected to do the impossible. It cannot perform the functions of other elements. It is idle to expect calcium in any form to be a satisfactory substitute for other elements, or for its application to compensate fully for the lack of intelligent soil management.

Thus the approach to the soil today is very different from what it was a few decades ago when the soil was considered mainly as a medium for plant growth. This approach, however, is far from universal among soil investigators. Unfortunately, many agricultural workers still look on the soil largely as a purveyor of plant nutrients.

The substantial progress that has been made in recent years has resulted from researches directed toward an understanding of the essential nature of the soil and of its properties and processes. But very much remains to be done. There is still great need in this field, as in many others of biological importance, for clarity of thinking and precision of statement. To understand the soil requires vastly more than rule-of-thumb procedure. To designate a soil process as a colloidal phenomenon is far from reducing the subject to clearly defined concepts, and to explain this or that as an adsorption process, when it is at least partly chemical precipitation (4), is likely to make confusion worse confounded. The problem requires the application of methods that were foreign to earlier soil investigators. Fortunately, however, an increasing number of soil scientists are striving to understand the elementary forces that are involved in the soil. The methods required are essentially analytical in kind, and the tools, the knowledge made available by the sciences of chemistry, physics, mineralogy, geology, and biology. The research worker, therefore, needs to be well trained in the fundamental sciences.

Finally, as the investigation of the soil has progressed, it has become increasingly evident that the soil is a complicated system. As was said in 1905 by the late Sir Daniel Hall, the soil "involves some of the most complex and difficult problems the world is ever likely to have to solve."

## REFERENCES

- (1) HENDRICKS, S. B. 1945 Base exchange of crystalline silicates. *Indus. and Engin. Chem.* 37: 625-630.
- (2) JENNY, H., AND OVERSTREET, R. 1938 Contact effects between plant roots and soil colloids. *Natl. Acad. Sci. Proc.* 24: 386-392.
- (3) KELLEY, W. P., ET AL. 1939 Comparative study of the colloids of a Cecil and a Susquehanna soil profile. *Soil Sci.* 47: 175-193.
- (4) KURTZ, T., DETURK, E. E., AND BRAY, R. H. 1946 Phosphate adsorption by Illinois soils. *Soil Sci.* 61: 111-124.
- (5) WIEGNER, G. 1912 Zum Basenaustausch in der Achererde. *Jour. Landw.* 60: 111-150, 197-222.

# EFFECT OF CERTAIN SOIL AND IRRIGATION TREATMENTS ON CITRUS CHLOROSIS IN A CALCAREOUS SOIL:

## I. PLANT RESPONSES

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Low vigor and low productivity of citrus associated with foliage chlorosis of various types have long been major problems in the culture of this crop in the United States and elsewhere. Much progress has been made in the control of specific types of chlorosis by the use of various trace elements such as zinc, manganese, and magnesium. However, a type of chlorosis, possibly related to iron and calcium metabolism and sometimes associated with manganese and zinc deficiencies as complicating factors (9, 10), is still a problem in the Southwest, particularly on the irrigated calcareous soils of Arizona and the arid inland valleys of California. Soils having a high soluble salt content produce trees of low vigor associated with a type of foliage chlorosis and necrosis in advanced stages (5). The studies reported here are concerned with a calcareous soil having a relatively low soluble salt content. In some instances high salinity of the soil is undoubtedly an additional complicating factor in the chlorosis problem on many of these calcareous soils.

In Arizona the studies of Burgess and Pohlman (2) were among the first to demonstrate that the incidence of chlorosis was related to the method of irrigation on a heavily silted, calcareous Yuma Mesa soil. Their studies indicated that the practice of maintaining the moisture level of soil near the field capacity much of the time was associated with low tree-vigor, chlorosis, and dieback. They found that marked improvement of this condition could be produced by making a heavy application of 5 or 6 acre-inches of water at each irrigation and allowing the soil to dry almost to the wilting coefficient between irrigations. They found that spraying the foliage with iron and manganese solutions produced only a transitory improvement in foliage color and that heavy application, in trenches, of manure mixed with superphosphate produced some improvement, but not comparable to that produced by the soil moisture control treatment. Later Finch *et al.* (9, 10) reported that the chlorosis of citrus prevalent on these calcareous soils appeared to be primarily an iron deficiency, or at least related to iron metabolism; but none of the control measures attempted were very effective. In a few instances zinc deficiency was also found. McGeorge (16) also studied the problem of chlorosis and decline of citrus in Arizona. He reported that he obtained some control of this disorder by heavy applications of

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sulfur, sulfuric acid, and a manure-phosphate combination. Leaf analyses indicated that chlorotic citrus leaves were abnormally low in calcium. Pot studies with various plants showed that a low level of calcium, iron, and manganese in the foliage is fairly characteristic of plants grown on alkaline-calcareous soils. Treatment of such soils with acidifying materials such as sulfur, sulfur plus manure, and sulfuric, nitric, or carbonic acid usually resulted in an increase in the amount of the elements calcium, manganese, and iron in the tissue. As a result of his studies, he advanced the hypothesis that the disorder is due to the high pH of the soil solution of alkaline-calcareous soils. This in turn reduces the availability of such essential nutrients as calcium, manganese, iron, and zinc. He did not attempt to rationalize this hypothesis with the soil moisture effect noted by Burgess and Pohlman (2).

Haas (11, 12) pointed out that lime-induced chlorosis is an important physiological disturbance of citrus on the calcareous soils in California. He suggested that the problem is intimately related to the moisture status and pH of the soil, which in turn are related to the physical structure of the soil and irrigation practice. He reported that, in certain experimental orchards, chlorosis was prevented by controlling the irrigation in such a way as to keep the soil as dry as was advisable and by addition of acid to the irrigation water. In some cases, very heavy applications of sulfur effectively controlled chlorosis.

The experiments reported here were designed to study further the relation of various soil and irrigation treatments to chlorosis of citrus on calcareous soils. In addition, the effect of such treatments on soil atmosphere composition was examined. These latter studies are summarized in another paper (20).

#### FOLIAGE SYMPTOMS ASSOCIATED WITH HIGH SOIL-MOISTURE CONTENT IN CALCAREOUS SOILS

Before the experiments with which this paper is primarily concerned are described and summarized, it seems desirable to describe the foliage symptoms which commonly occur on some bearing Marsh grapefruit plantings on the calcareous soils of the Coachella Valley, and the relation of these symptoms to irrigation practice. In a block of young bearing Marsh grapefruit at the U. S. Date Garden, Indio, California, certain plots are maintained at a high moisture level throughout the year by frequent irrigation, and another series of plots is irrigated when most of the available moisture from the previous irrigation has been removed from the top 2 feet of soil. In the winter months, foliage chlorosis is much more pronounced in the high-moisture plots than in the low-moisture. In summer, scarcely any difference can be discerned by casual inspection of the plots, and there is little chlorotic foliage on any of the trees, regardless of irrigation treatment. In the early winter months, the outside foliage (youngest) from the high-moisture plots tends to exhibit the range of symptoms illustrated in figure 1, particularly on the most exposed side of the tree. The branch on the left shows mild chlorosis and venation, and the one on the right shows more severe chlorosis of the terminal shoot leaves. Note that the older basal leaves

from a previous "flush" are not chlorotic. The chlorotic foliage illustrated originated sometime during the summer or early fall of 1944, and the photograph was taken in March, 1945. Shaded foliage (older) on the inside of the tree may also exhibit chlorosis, but usually the symptoms are less pronounced than on exposed leaves. The branches in figure 2 illustrate the range of chlorosis usually found on inside leaves. Note also that these tend to be somewhat narrower and more pointed than corresponding exposed leaves. Figure 3 illustrates a more advanced stage of chlorosis which develops on many of the chlorotic terminal shoots in late winter and early spring. Note the brown discolorations which



FIG. 1. MILD CHLOROSIS (LEFT) AND SEVERE CHLOROSIS (RIGHT) OF EXPOSED FOLIAGE OF MARSH GRAPEFRUIT ASSOCIATED WITH HIGH SOIL MOISTURE CONTENT IN CALCIAREOUS SOIL

occur most prominently on the most exposed portion of the chlorotic leaf. At this stage, many leaves abscise, so that only the defoliated terminal twigs remain, as illustrated by the branch on the right. In some instances these defoliated twigs die back; in others, weak, chlorotic shoots develop from many of the axillary buds.

With the onset of warmer weather in the spring, vegetative and flowering shoots push out, and the new, developing foliage tends to exhibit slight chlorosis and venation before it becomes fully developed. By midsummer, chlorosis becomes much less intense, because most of the new leaves become fully green, and the older leaves of the previous "flush" which showed moderate to severe



FIG. 2 MILD (LEFT) AND SEVERE (RIGHT) CHLOROSIS OF SHADED FOLIAGE OF MARSH GRAPEFRUIT ASSOCIATED WITH HIGH SOIL MOISTURE CONTENT IN CALCIAROUS SOIL



FIG. 3 SEVERE CHLOROSIS, BROWN DISCOLORATION AND DEFOLIATION OF EXPOSED TERMINAL TWIGS OF MARSH GRAPEFRUIT ASSOCIATED WITH HIGH SOIL MOISTURE CONTENT IN CALCIAROUS SOIL

chlorosis in winter have either fallen off or partially recovered. The only remaining chlorotic leaves are on the inside shoots. These do not seem to abscise in the early spring or recover in the summer to the same degree as do exposed leaves. The difference between high-moisture and low-moisture plots is in the prevalence and severity of chlorosis in winter and early spring. In the low-moisture plots, far fewer terminal shoots show severe chlorosis, and much less abscission of foliage takes place.

#### PLAN OF EXPERIMENT

In the spring of 1942, 2-year-old Marsh grapefruit trees were planted in plots on a friable calcareous soil, typical of much of the soil in the Coachella Valley of California. The profile of this soil (classified as Indio very fine sandy loam) is variable but is predominantly very fine sandy loam containing numerous sharply defined strata of silt loam to silty clay loam which vary greatly in location and thickness. Analysis of soil samples taken from these plots before planting showed that the first foot contained 4.8 to 8.7 per cent of carbonate calculated as calcium carbonate, according to the volumetric A. O. A. C. method (1), and between 150 and 300 p.p.m. of soluble salts when determined by measuring the resistance of a soil paste by the old U. S. Bureau of Soils method (8). Salt concentrations in the soil solution at field capacity or at a lower moisture content would be appreciably greater. The corresponding second-foot samples contained 11.0 to 12.4 per cent of calcium carbonate and between 100 and 200 p.p.m. of soluble salts.

Young citrus trees were set out in a split-plot design, which made it possible to compare eight soil treatments at two irrigation levels, using seven replications. Holes 4 feet wide by 6 feet long and 2 feet deep were dug for each of the 112 subplots. Certain of the soil treatments were mixed thoroughly with the excavated soil; then the holes were refilled and planted with two young Marsh grapefruit trees each. The soil treatments were as follows:

NO.	TREATMENT	METHOD OF APPLICATION	AMOUNT (LBS.) PER SUBPLOT
A	Steer manure	Mixed with soil	80
B	Chopped alfalfa hay	Mixed with soil	80
C	Triple superphosphate	Mixed with soil	20
D	Soil compacted		
E	None, check		
F	Ammonium sulfate	Broadcast on surface of plot 3 times a year	5*
G	Sulfur	Mixed with soil	60
H	Magnetite ( $Fe_3O_4$ )	Mixed with soil	15

\* Per application.

The wet-plot series was irrigated with 2 to 3 acre-inches of water at weekly intervals in the summer months and every 2 or 3 weeks in the winter months. The dry plots were irrigated with a similar amount of water every 2 or 3 weeks in the

summer of 1942 and every 4 to 6 weeks in the summer of 1943. In the winter months they were irrigated every 6 to 8 weeks. Adjacent wet and dry subplots were separated by a border or dike 4 feet wide at the base. Blocks of subplots were separated by a strip 10 feet wide which was irrigated in the same manner as the dry plots.

#### EXPERIMENTAL METHODS

Plant responses were evaluated by making periodic and systematic evaluations of the degree of chlorosis displayed by the foliage and the amount of new growth produced. In addition, foliage samples were obtained from shoots of a definite age (that is, originating in the same "flush"), and only leaves of a definite position on the shoot were selected for sampling. The leaf samples were dried at 48° C., weighed, ground to a very fine powder, and analyzed for chlorophyll and organic nitrogen content.

Chlorophyll was determined by adapting the method of Petering *et al.* (19) as follows:

Approximately 0.5 gm. of leaf tissue, dried at 48° C. and ground to a very fine powder in a small Wiley mill, is placed in a 30-ml. Gooch-type crucible with fritted glass disc of fine porosity, brought to constant weight at 48° C., weighed, then extracted with 180 ml. of 80 per cent ethyl alcohol. The extraction process requires approximately 18 hours and is accomplished by inverting above the crucible a 250-cc. stoppered Florence flask containing the alcohol. The stopper of the inverted flask is fitted with two protruding glass tubes, one about 2 to 3 mm. longer than the other. This automatic arrangement keeps the level of the solvent in the crucible virtually constant until all the solvent is gone. The extraction is carried out in a photographic darkroom. The filtrate is collected in a 200-ml. volumetric flask, made to volume with alcohol, and the chlorophyll content estimated colorimetrically according to Petering's procedure.

It was found that, under the conditions of this experiment, this procedure gave extraction efficiency comparable to that obtained by the method prescribed by Petering *et al.*, and required much less time, since 12 to 24 samples can be extracted at one time. The probable error of a single determination by this technique is approximately 1.5 per cent.

Total organic nitrogen and ammoniacal nitrogen were determined by the standard Kjeldahl-Gunning-Arnold method (1).

#### EXPERIMENTAL RESULTS

No differences among any of the treatments were apparent until late Autumn in 1942. By December of that year there was striking difference in foliage color between the wet and the dry series of subplots. The predominant leaf patterns on most of the wet plots suggested iron and nitrogen deficiencies. Chlorosis tended to be most intense on the youngest leaves. Treatment of a few of these chlorotic leaves with a 0.2 per cent solution of Fe SO<sub>4</sub> produced the characteristic partial "greening up" of the leaves described by other workers (6, 9, 10). These symptoms were far less prevalent on most of the dry plots, but symptoms suggesting mild manganese deficiency were fairly prevalent.

Systematic observations indicated that there were consistent responses to some of the soil treatments. The trees in the superphosphate-treated plots, without exception in both wet and dry series, produced marked symptoms closely resembling mottle leaf (5), a zinc deficiency. These were the only plots in which this was the predominant leaf pattern. Chapman *et al.* (4) have reported that the degree of mottling of citrus can be strongly increased by increasing the phosphorus content of a low-zinc culture medium. The sulfur-treated trees showed slight symptoms suggesting sulfate toxicity as described by Chapman and Kelley (5), and in the first summer they made very poor growth, with very small leaves. During the second summer, salt-toxicity symptoms were virtually absent, and leaves were normal in size. The hay-treated plots made exception-

TABLE 1

*Effect of soil and irrigation treatments on chlorosis index<sup>1</sup> of foliage of young Marsh grapefruit trees*

IRRIGATION TREATMENTS	SOIL TREATMENTS								Mean
	A Manure	B Hay	C Phos- phate	D Com- pacted	E Check	F N	G Sulfur	H $\text{Fe}_3\text{O}_4$	
<i>April 6, 1943</i>									
Wet.....	30	11	43	19	23	15	13	22	22.00
Dry .....	14	5	28	7	7	9	8	6	10.50
<i>July 8, 1943</i>									
Wet.....	2	1	29	5	6	0	0	7	6.25
Dry.....	1	0	14	3	2	5	0	0	3.13

<sup>1</sup> Obtained by classifying each of the 224 trees (2 in each of 112 subplots) in one of five scored categories with respect to the incidence of chlorotic foliage. The scored categories were as follows: 0 = none to trace; 1 = slight; 2 = moderate; 3 = severe; and 4 = very severe. Trees placed in the very severe category were judged to have 50 per cent or more of the leaves showing distinct symptoms of chlorosis. The chlorosis index was obtained by simply adding the individual scores for the 14 trees in the seven subplots devoted to each treatment.

ally good growth, producing somewhat less chlorosis in both wet and dry plots than the corresponding check plots. Nitrogen, magnetite, and steer manure, on the other hand, were all ineffective in reducing the prevalence of chlorotic leaves in the wet plots.

With the return of warm weather in the early summer of 1943, the differences in foliage chlorosis between wet and dry plots became less pronounced, so that by midsummer it was scarcely discernible by inspection. In fact, there was very little chlorotic foliage in any of the subplots, regardless of irrigation or soil treatment, with the exception of the phosphorus-treated plots, all of which continued to show pronounced symptoms resembling mottle leaf, or zinc deficiency. The data presented in table 1 represent an attempt to summarize the foregoing observations numerically.

Foliage samples were collected on April 6, 1943, when the difference in chlorosis between wet and dry plots was still pronounced, and again on July 8, when the difference was not apparent to the eye. The dry weights of the leaf samples composited from the two trees in each subplot were determined and then the samples were analyzed for chlorophyll and total organic and ammoniacal nitrogen. The data presented in table 2 indicate that at the time of the April sampling there was no significant effect of irrigation on the dry weight of leaves

TABLE 2  
*Effect of soil and irrigation treatments on weight of leaves from young Marsh grapefruit trees*  
Weights in grams per leaf, dry basis

SOIL TREATMENT PLOTS MEAN <sup>1</sup> WT.								IRRIGATION SUBPLOTS MEAN <sup>2</sup> WT.	
A Manure	B Hay	C Phosphate	D Com- pacted	E Check	F N	G Sulfur	H Fe <sub>2</sub> O <sub>3</sub>	Wet	Dry
<i>Samples collected April 6, 1943</i>									
0.187	0.196	0.199	0.194	0.197	0.179	0.146**	0.195	0.184	0.189
<i>Samples collected July 8, 1943</i>									
0.241	0.258	0.268	0.245	0.245	0.244	0.244	0.277	0.267**	0.238**

<sup>1</sup> Mean of 14 samples—one pair of irrigation subplots in each of the seven blocks.

<sup>2</sup> Mean of 56 samples—eight irrigation subplots in each of seven blocks.

\*\* Significant at the 1 per cent level.

#### *Indications of statistical calculations*

(Analysis of variance)

#### *April 6 samples:*

1. There is a highly significant effect of soil treatments, but the effects of irrigation treatments and the interaction between irrigation and soil treatments are not significant.
2. The least difference for significance between any two soil treatment means is 0.036 at the 0.01 level, and 0.027 at the 0.05 level. Thus, the leaves from the sulfur plots were significantly smaller (on the basis of dry weight) than those from the check plots. None of the other soil treatments produced leaves significantly larger or smaller than the check treatment.

#### *July 8 samples:*

1. The effect of soil treatments is not significant, but the effect of irrigation treatments is highly significant. There is no significant interaction between these two factors, however.

and no effect of soil treatments except in the case of the sulfur-treated plots. These had significantly smaller leaves, based on dry weight, than the check plots. The July 8 samples showed no apparent effect of soil treatments on the dry weight of the foliage, but the effect of irrigation treatments was highly significant, the wet plots producing somewhat heavier leaves than the dry plots.

The data presented in table 3 indicate that in April there was a significant effect of both soil and irrigation treatments on the percentage of organic and ammoniacal

nitrogen in the dry matter of the foliage. The manure, hay, and nitrogen treatments produced foliage having significantly more nitrogen than the check treatment, and the sulfur treatment showed less nitrogen than the check treatment. The foliage from the dry series of subplots had approximately 13 per cent more nitrogen in relation to total dry matter than the wet series of subplots. By midsummer (July 8) there was no longer a significant effect of either soil or irrigation treatments on the nitrogen content of the foliage.

TABLE 3

*Effect of soil and irrigation treatments on organic nitrogen content of foliage of young Marsh grapefruit trees*

N, in percentage of dry matter

SOIL TREATMENT PLOTS MEAN <sup>1</sup> N								IRRIGATION SUBPLOTS MEAN <sup>2</sup> N	
A Manure	B Hay	C Phos- phate	D Com- pacted	E Check	F N	G Sulfur	H Fe <sub>2</sub> O <sub>3</sub>	Wet	Dry
<i>Samples collected April 6, 1943</i>									
2.99**	2.89**	2.84	2.80	2.72	2.99**	2.46**	2.78	2.63**	2.98**
<i>Samples collected July 8, 1943</i>									
2.65	2.61	2.71	2.57	2.61	2.75	2.57	2.62	2.64	2.63

<sup>1</sup> Mean of 14 samples—paired wet and dry subplots receiving the same soil treatment in the seven blocks.

<sup>2</sup> Mean of 56 samples—eight subplots receiving the same irrigation treatment in seven blocks.

\*\* Significant at the 1 per cent level.

*Indications of statistical calculations  
(Analysis of variance)*

*April 6 samples:*

1. The general effects of both soil and irrigation treatments are highly significant.
2. The least difference for significance between any two soil treatment means is 0.21 at the 0.01 level and 0.16 at the 0.05 level. Thus the manure, hay, and nitrogen plots have significantly more nitrogen in the foliage than do the check plots, and the sulfur plots have significantly less.

*July 8 samples:*

1. On this sampling date there was no significant effect of either the fertilizer or the irrigation treatments.

The data presented in table 4 indicate the effect of soil and irrigation treatments on the percentage of chlorophyll in the dry matter of the foliage samples harvested April 6. The data show that there was a significant difference between all the pairs of irrigation treatments except the sulfur plots. In the case of the soil treatments, the data indicate that in the wet series of subplots, the hay and sulfur treatments had significantly more, and the phosphate treatment less, chlorophyll than the corresponding check treatment. In the dry series, only the

TABLE 4

*Effect of soil and irrigation treatments on the chlorophyll content of foliage of young Marsh grapefruit trees*

Samples collected April 6, 1943—chlorophyll, in percentage of dry matter

IRRIGATION TREATMENTS	SOIL TREATMENTS MEAN <sup>1</sup> CHLOROPHYLL								MEAN <sup>2</sup>
	A Manure	B Hay	C Phos- phate	D Com- pacted	E Check	F N	G Sulfur	H $\text{Fe}_2\text{O}_4$	
Wet.....	0.237	0.346**	0.216*	0.270	0.270	0.309	0.393**	0.283	0.290**
Dry.....	0.401	0.457*	0.407	0.391	0.406	0.421	0.419	0.380	0.410**

<sup>1</sup> Mean of seven samples—one subplot in each of seven blocks.

<sup>2</sup> Mean of 56 samples—eight irrigation subplots in each of seven blocks.

\* Significant at the 5 per cent level.

\*\* Significant at the 1 per cent level.

*Indications of statistical calculations*

(Analysis of variance)

1. The effect of both soil and irrigation treatments is highly significant.
2. The interaction between soil and irrigation treatments is highly significant.
3. The least difference for significance between any two soil treatment means (having the same irrigation treatment) is 0.072 per cent at the 0.01 level, and 0.054 per cent at the 0.05 level. Thus in the wet-plot series, treatments B, C, and G differ significantly from the check treatment, and in the dry-plot series, only the B treatment differs significantly from the check.
4. The least difference for significance between any of the pairs of irrigation treatments is 0.057 per cent at the 0.01 level, and 0.043 per cent at 0.05. Thus there is a significant difference between all the pairs of irrigation treatments, except G (sulfur).

TABLE 5

*Effect of soil and irrigation treatments on chlorophyll content of foliage of young Marsh grapefruit trees*

Samples collected July 8, 1943—chlorophyll, in percentage of dry matter

SOIL TREATMENT PLOTS MEAN <sup>1</sup> CHLOROPHYLL								IRRIGATION SUBPLOTS MEAN <sup>2</sup> CHLOROPHYLL	
A Manure	B Hay	C Phos- phate	D Com- pacted	E Check	F N	G Sulfur	H $\text{Fe}_2\text{O}_4$	Wet	Dry
0.233	0.255**	0.211	0.215	0.224	0.249*	0.291**	0.234	0.224	0.251

<sup>1</sup> Mean of 14 samples—paired wet and dry subplots receiving the same soil treatment in the seven blocks.

<sup>2</sup> Mean of 56 samples—eight subplots receiving the same irrigation treatment in the seven blocks.

\* Significant at the 5 per cent level.

\*\* Significant at the 1 per cent level.

*Indications of statistical calculations*

(Analysis of variance)

1. The effect of both soil and irrigation treatments is highly significant.
2. The least difference for significance between any two soil treatment means is 0.030 at the 0.01 level and 0.022 at the 0.05 level. Thus the chlorophyll contents of the foliage from the hay, nitrogen, and sulfur plots were significantly greater than that of the check plots.

hay treatment had significantly more chlorophyll than the corresponding check treatment.

The data summarized in table 5 show the chlorophyll content of the foliage in midsummer. Since the interaction between the soil and irrigation treatments was not significant at this time, the means of the paired wet and dry subplots were combined to facilitate comparison of the soil treatments. Statistical calculations indicate that the over-all effect of irrigation treatment (the difference between the irrigation subplot means) was highly significant, despite the fact that scarcely any consistent effect of irrigation treatments on chlorosis was apparent upon visual inspection (see table 1). It is to be noted that in April, when a marked difference in chlorosis was visible even by casual inspection, the dry series of subplots had approximately 40 per cent more chlorophyll than the wet series of subplots, whereas in July, the dry series had only about 12 per cent more. If chlorophyll is calculated on a leaf-unit basis (milligrams per leaf), the corresponding difference in April is about 45 per cent, whereas in July it is less than 1 per cent. Thus the larger leaves harvested from the wet plots in July seem to have about the same absolute quantity of chlorophyll but a slightly smaller concentration in relation to total dry matter. It is of interest to note that in July the sulfur plots, while producing plants of low vigor with symptoms suggesting salt toxicity, produced foliage that was higher in chlorophyll content on both a percentage and an absolute basis than comparable foliage from check plots. The same condition prevailed in April in the wet series of subplots but not in the dry. Thus it appears that the very heavy application of sulfur changed the soil condition in some way so that chlorosis was reduced (or chlorophyll synthesis was favored), but other factors unfavorable for vigorous growth were introduced.

#### DISCUSSION

This short-time experiment did not indicate any detriment to growth and vigor of young citrus trees resulting from overirrigation, despite the development of considerable winter chlorosis associated with this treatment. Had the experiment continued for a longer period, it is probable that severe winter chlorosis would eventually have reduced tree vigor. Field observations in the more arid citrus-growing sections of the Southwest indicate that a seasonal fluctuation in the prevalence of chlorotic foliage is common in many citrus plantings. The data presented here and the experience of others (2, 6, 12) indicate that maintaining soil moisture at a high level by frequent irrigation tends to accentuate this phenomenon associated with calcareous soils. In extreme cases, when severe chlorosis persists year after year, vigor and yield may be reduced seriously. It is not known how much the yielding capacity of trees exhibiting moderate symptoms in the winter months is affected.

Chlorosis of many perennial fruit and ornamental plants on calcareous soils has long been an important agricultural problem which has received much study. Some progress has been made, but still relatively little is known of the fundamental cause or causes, and practical control measures have not yet been devised in many of the problem areas. Since good discussions of the present status of

research on this problem may be found in recent papers (13, 15, 17, 21), no detailed review is presented here. Suffice to say that the question as to whether this type of chlorosis is due to unavailability of iron in the soil or to a lack of mobility of this element in the plant tissue has received much attention. Lindner and Harley (15) favor the hypothesis that high-lime soil produces a condition within the plant tissue which renders the iron taken up by the roots unavailable for normal functions. Jacobson (13) found good correlation between the iron and chlorophyll content of leaves from plants showing symptoms of lime-induced chlorosis. Thorne and Wallace (21) suggest that their work indicates that unavailability of iron in both soil and plant tissue may be involved, and that the unavailability in both cases is related in some way to the state of oxidation of the Fe ion. Our findings suggest that any hypothesis advanced to explain the cause of "lime-induced chlorosis" should take into consideration the seasonal fluctuation in severity noted with nondeciduous plants such as citrus.

The studies of Cameron (3), working with Valencia oranges grown at Riverside, California, indicate that there is a very marked accumulation of starch in most tissues of citrus during the winter months, particularly in the leaves and in the phloem of the small roots. In Arizona, Martin (18), working with Marsh grapefruit, showed that starch was virtually absent from leaves in the hottest months but accumulated to a marked degree in winter. The starch-sugar ratio also varied profoundly with the season. Thus, it can be seen that if marked seasonal changes in the carbohydrate status of citrus tissues can occur, it is reasonable to assume that other significant changes in tissue composition and metabolism may occur also. Some of these changes may have a profound effect on the utilization of iron, and possibly other elements, in relation to chlorophyll formation. These seasonal changes within the tissue are associated with changes in environmental factors, such as temperature of soil and air, light intensity, and length of day. One or more of these factors may be closely related to the failure of the plant to take up iron or some other element essential to the normal utilization of iron. For example, Jones (14) has shown that chlorosis of gardenias (presumably on noncalcareous soil) may be controlled under greenhouse conditions simply by maintaining the soil temperature above 20°C., and induced by maintaining the temperature below 18°C. Working with a full nutrient solution in sand culture, Davidson (7) concluded that increases in light intensity and in length of day, as well as increases in air temperature, tend to offset chlorosis of gardenia foliage and retardation of growth caused by low root temperatures. Chapman, *et al.* (4) have shown that light intensity affects the incidence of mottle-leaf in citrus plants grown in a zinc-deficient culture.

These experiments with gardenias and citrus suggest that light intensity and temperatures of air and soil are factors which may be involved in the winter chlorosis of citrus noted in the calcareous soils of the arid Southwest. Observations in the Coachella Valley indicate that winter chlorosis of citrus is rare on trees grown in the shade of date palms. In orchards not interplanted with dates, chlorosis is more severe on the south than on the north side of the trees. This has also been reported by others (6). However, the fact that the chlorophyll

content of the foliage may be reduced by maintaining a high moisture level in the soil and increased by mixing chopped hay or sulfur in the soil suggests that the primary causal factor is low availability of one or of a group of nutrient elements taken up by the roots. It is possible that when environmental factors become less favorable for vigorous accumulation of difficultly available nutrients by roots or for rapid translocation and/or utilization within the plant, the deficiency is transformed from an incipient stage to a visible chlorosis.

The effects of soil moisture level, certain soil treatments, and soil temperature on soil aeration in relation to citrus chlorosis will be considered in another closely related paper (20).

#### SUMMARY

Young Marsh grapefruit trees were set out on a calcareous soil in a split-plot design for the purpose of evaluating the effect of various soil treatments and two moisture levels on the prevalence of foliage chlorosis. There was a striking interaction of season and moisture treatments on the incidence of chlorosis. During the summer months, virtually no chlorosis occurred on any of the plots regardless of moisture treatments, but during winter and early spring chlorosis increased very markedly on the wet (high-moisture) plots and only slightly on the dry (normal-moisture) plots. On the other hand, most of the wet trees made more growth and had somewhat larger leaves (greater dry weight) than the dry trees. The behavior of these young experimental trees was somewhat at variance with the behavior of bearing commercial orchards in this region. Frequently chlorosis persists throughout the summer on inside shoots on larger, bearing trees.

There were also some responses to some of the soil treatments. Trees treated with a very heavy application of triple superphosphate produced a profusion of foliage symptoms suggesting zinc deficiency, which persisted even in summer in both moisture treatments. In no other treatment was this the predominating leaf pattern. Very heavy applications of sulfur induced poor growth, very small leaves, and foliage symptoms suggesting salt toxicity. The plots treated with a large amount of chopped alfalfa hay made exceptionally good growth and possibly developed somewhat less chlorosis in winter than any of the other treatments. Very heavy applications of nitrogen, steer manure, or magnetite (an iron oxide ore) did not prevent the occurrence of considerable chlorosis in the high-moisture plots during winter and early summer.

Analysis of foliage samples obtained in April (when the difference in chlorosis between the two moisture treatments was marked) indicated that, with one exception, neither soil treatments nor irrigation treatments had any significant effect on the dry weight of leaves. Foliage from sulfur-treated trees was smaller than that from any other soil treatment. By the following July, however, there was no longer any effect of soil treatments, but the leaves from the high-moisture plots had a greater dry weight than those from the normal-moisture plots.

These same foliage samples were analyzed for percentage of total organic nitrogen. The data obtained from the April samples indicated about 13 per cent

more nitrogen in the dry series of plots than in the wet, but in July the irrigation treatments showed no effect. The April samples also indicated that, in the wet series, that significantly more nitrogen occurred in the foliage from plots treated with manure, hay, or nitrogen and significantly less in the foliage from the sulfur-treated plots. By July, however, there was no longer a significant effect of soil treatments on nitrogen content of leaves.

Additional analyses of the April samples from the wet series for chlorophyll content indicated that hay and sulfur significantly increased and phosphorus decreased the chlorophyll content of foliage in relation to dry weight. In the dry series, only hay increased the chlorophyll content of foliage. When the overall effect of moisture is considered, the dry plots had about 40 per cent more chlorophyll than the wet plots in April, but by July the difference was only about 12 per cent. When expressed on an absolute basis (milligrams of chlorophyll per leaf), the difference in April was about the same as the percentage difference, but in July there was no difference at all.

Chlorophyll analyses of the July samples indicated that hay, nitrogen, and sulfur significantly increased the concentration of this constituent in the foliage.

#### REFERENCES

- (1) Association of Official Agricultural Chemists 1940 Official and Tentative Methods of Analysis, ed. 5. Washington, D. C.
- (2) BURGESS, P. S., AND POHLMAN, G. G. 1928 Citrus chlorosis as affected by irrigation and fertilizer treatments. *Ariz. Agr. Exp. Sta. Bul.* 124: 1-232.
- (3) CAMERON, S. H. 1932 Starch in the young orange tree. *Proc. Amer. Soc. Hort. Sci.* 29: 110-114.
- (4) CHAPMAN, H. D., VANSELOW, A. P., AND LIEBIG, G. F. 1937 The production of citrus mottle-leaf in controlled nutrient cultures. *Jour. Agr. Res.* 55: 365-379.
- (5) CHAPMAN, H. D., AND KELLEY, W. P. 1943 The mineral nutrition of citrus. [WEBBER, H. J., AND BATCHELOR, L. D. (Editors) *The Citrus Industry*, Vol. 1: 749-766. The University of California Press, Berkeley.]
- (6) CHAPMAN, H. D., BROWN, N. S., AND RAYNER, D. S. 1945 Nutrient deficiencies of citrus. *Calif. Citrogr.* 30: 162-163.
- (7) DAVIDSON, O. W. 1941 Effect of temperature on growth and flower production of gardenias. *Proc. Amer. Soc. Hort. Sci.* 39: 387-390.
- (8) DAVIS, R. O. E., AND BRYAN, H. 1910 The electrical bridge for the determination of soluble salts in soils. *U. S. Dept. Agr. Bur. Soils Bul.* 61: 1-36.
- (9) FINCH, A. H., ALBERT, D. W., AND KINNISON, A. F. 1938 A chlorotic condition of plants in Arizona related to iron deficiency. *Proc. Amer. Soc. Hort. Sci.* 30: 431-434.
- (10) FINCH, A. H., ALBERT, D. W., AND KINNISON, A. F. 1934 Progress on the control of citrus chlorosis or decline. *Proc. Amer. Soc. Hort. Sci.* 32: 20-33.
- (11) HAAS, A. R. C. 1932 Some nutritional aspects in mottle-leaf and other physiological diseases of citrus. *Hilgardia* 6: 483-559.
- (12) HAAS, A. R. C. 1942 Lime-induced chlorosis of citrus in relation to soil factors. *Plant Physiol.* 17: 27-51.
- (13) JACOBSON, L. 1945 Iron in the leaves and chloroplasts of some plants in relation to their chlorophyll content. *Plant Physiol.* 20: 233-245.
- (14) JONES, L. H. 1938 Relation of soil temperature to chlorosis of gardenias. *Jour. Agr. Res.* 57: 611-621.

- (15) LINDNER, R. C., AND HARLEY, C. P. 1944 Nutrient interrelations in lime-induced chlorosis. *Plant Physiol.* 19: 420-438.
- (16) McGEORGE, W. T. 1936 Some aspects of citrus tree decline as revealed by soil and plant studies. *Ariz. Agr. Exp. Sta. Tech. Bul.* 60.
- (17) MAGISTAD, O. C. 1945 Plant growth relations on saline and alkali soils. *Bot. Rev.* 11: 181-230.
- (18) MARTIN, W. E. 1942 Physiological studies of yield, quality and maturity of Marsh grapefruit in Arizona. *Ariz. Agr. Exp. Sta. Tech. Bul.* 97: 1-48.
- (19) PETERING, H. G., HOLMAN, W., AND HIBBARD, R. P. 1940 Determination of chlorophyll and carotene in plant tissue. *Indus. and Engin. Chem., Analyt. Ed.* 12: 148-151.
- (20) REUTHER, W., AND CRAWFORD, C. L. Effect of certain soil and irrigation treatments on citrus chlorosis in a calcareous soil: II. Soil atmosphere studies. To appear in *Soil Science*.
- (21) THORNE, D. W., AND WALLACE, A. 1944 Some factors affecting chlorosis on high-lime soils: I. Ferrous and ferric iron. *Soil Sci.* 57: 299-312.



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